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ORMOND ROURKE BUTLER
1877-1940

I. E. MELHUS

Dr. Ormond Rourke Butler was head of the Department of Botany of the University of New Hampshire and Botanist of the New Hampshire Agricultural Experiment Station from 1912 until the time of his death.

The copper fungicides received Dr. Butler's best efforts for many years, and his contributions bear evidence of his originality and thoroughness. It was natural that his researches on the properties of fungicides should lead him to give special attention to the control of apple and potato diseases. In all his research he worked carefully, feeling that no price was too great to pay for accuracy. He thoroughly understood his field and had outstanding ability in organizing his research. Dr. Butler read the literature to such an extent that it became a part of him. He refused to be drawn into any argument and enjoyed a clear exposition of another person's point of view. Favor and prejudice never were permitted to influence his findings in research. All scientific data obtained through his investigations could be accepted without any question as to their authenticity.

Although he seldom was ill, he was not robust. Throughout his career as a scientist, however, he worked tirelessly. He did not have a wide circle of friends but rather a few very loyal ones. His sterling character was manifested by his admiration and respect for his father and mother and his loyalty to his friends. He was generous, sympathetic, and retiring almost to his own detriment.

Dr. Butler had several hobbies. He was greatly interested in gardening, particularly in the growing of rhododendrons. Another hobby was that of cooking, which began with a study that he made on the cooking qualities of potatoes. It is reported that he left three notebooks filled with data and recipes.

Symphonic music, the ballet, and opera were among his diversions. He had a thorough command of the French language and prized his collection of French books. It was always his dream that he might return to visit in France and Switzerland, where he spent most of his youth.

Ormond Rourke Butler was born in Melbourne, Australia, on August 14, 1877. He was the elder of two sons of Thomas and Mary Anne (Rourke) Butler. His mother was the daughter of Henry Rourke, a contractor and cattleman in Australia. In 1895 he received the diploma of the Institute Nationale Agricole, Lausanne, Switzerland, where he completed the equivalent of our grammar and high school studies. That same year he came to the United States and was granted citizenship fourteen years later. From 1895 to 1900 he was viticulturist-horticulturist in California. He entered the University of California where he received the degree of Bachelor of

Science and, following this, the degree of Master of Science, in 1905. From 1906 through 1908 he was assistant at the Southern California Pathological Laboratory at Whittier, California. Thereafter he pursued graduate work at Cornell University, where he received the degree of Doctor of Philosophy in 1910. He then became research instructor in the Department of Horticulture at the University of Wisconsin. From 1912 until the time of his death he was head of the Department of Botany of the University of New Hampshire and Botanist of the New Hampshire Agricultural Experiment Station. Dr. Butler died on October 24, 1940. He is survived by two sons,

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Robert E. and Alexander R. Butler. His wife, the former Roberta Van Horn, a graduate of North Dakota State College, preceded him in death on March 8, 1929.

Dr. Butler was a charter member of The American Phytopathological Society, a member of the American Society of Agronomy, a Fellow of the American Association for the Advancement of Science, and a member of Sigma Xi and Phi Kappa Phi.

At the time of his death Dr. Butler was engaged in several experiment station research projects, one of which was "Control of Apple Scab." His data and observations on this problem are now being prepared for publication. Other investigations included effect of temperature and other environ-

mental factors on the expression of symptoms of potato mosaic, spray injury by lime-sulphur, effect of temperature on potatoes in storage, control of bitter pit of apple, peony diseases, and the control of poison ivy.

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THE INHERITANCE OF RESISTANCE TO USTILAGO NUDA[1]

J. E. LIVINGSTON

(Accepted for publication July 17, 1941)

INTRODUCTION

Flower-infecting loose smut, caused by *Ustilago nuda* (Jens.) K. and S., is one of the most widely recognized diseases of barley in Missouri. The modified hot-water treatment (1) for destroying the dormant mycelium of *U. nuda* within the barley seed is difficult to apply, and, even under carefully controlled conditions, may cause some damage. It is desirable, therefore, to have available varieties that are resistant to the attack of this fungus and adapted to Missouri conditions. During the course of the breeding program to develop an adapted variety of winter barley resistant to *U. nuda*, an attempt was made to determine the behavior of the inheritance of resistance to this smut in various barley hybrids, and the linkage relations between the factors for resistance, hoods, six-row heads, and winter habit.

Zeiner (10) studied 8 crosses between barley varieties showing various degrees of susceptibility to *Ustilago nuda*. Resistance appeared to be dominant and controlled by a single factor, although the evidence was inconclusive. When two susceptible varieties were crossed there was no indication of transgressive segregation toward greater resistance.

Nammacher (5) confirmed many of Zeiner's results; the uncertainty of the infection method, however, made it difficult to obtain an accurate analysis of the factors governing the reaction of the F_3 progenies.

The low percentage of smutted heads resulting from artificial inoculation with chlamydospores has been one of the greatest handicaps in varietal testing for resistance to *Ustilago nuda*. Methods involving the clipping or spreading of the glumes have lowered the vitality of the seed (8, 10). Tapke (8) and Moore (4) have developed methods that give fairly good infection with this loose smut of barley and ordinarily produce plump and viable seed from inoculated flowers.

MATERIALS AND METHODS

In comparative tests the partial-vacuum method of inoculation described by Moore (4) gave the most satisfactory results and was used throughout this investigation. The flowers at the 3 terminal and basal joints of the rachis were removed and the remainder of the head inoculated from 12 to 24 hours after anthesis of the terminal flowers. The vacuum was supplied by making 20 strokes with an automobile pump having a $1\frac{3}{4}$-inch bore.

[1] The subject matter of this paper was submitted to the faculty of the Graduate College of the University of Missouri in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express his appreciation to Dr. C. M. Tucker for his supervision and criticism during the course of this investigation and for his suggestions in the preparation of the manuscript.

Inoculated plants were placed for 72 hours, in an incubation chamber, in the greenhouse with a humidity range of 85 to 95 per cent and a temperature range of 15 to 30° C. Night temperatures usually were maintained between 15° and 18°, and, during the daytime, between 20° and 22° C., except in late spring, when they frequently reached 30° C. Microscopic examination of the stigmas showed spore germination under these conditions in less than 24 hours.

The inoculum supply was built up and maintained on Missouri Early Beardless winter barley from a single infected head of the same variety. Spore structure and type of germination proved it to be *Ustilago nuda*. Mature smutted heads were collected and stored, in a refrigerator, from 30 to 60 days at 3° to 5° C. Germination of the spores was always tested before applying them, and only samples with a germination of 50 per cent or higher were used. The spore suspension used as inoculum consisted of approximately 50 mg. of chlamydospores of *U. nuda* in 60 ml. of tap water.

Inoculated seed was germinated in the laboratory and planted in paper plant bands, where they were kept until they were approximately 3 inches high, and then were transplanted to the field. This procedure reduced the seedling mortality loss of infected material.

Data on the susceptibility of the parent varieties to the Missouri Early Beardless collection of *Ustilago nuda* are presented in table 1. There was a fairly large variation in the limits of susceptibility, even though care was taken to maintain uniform inoculation and incubation conditions. Total infection in the susceptible varieties was not obtained. This may be due to differing varietal susceptibility or to some fault in the inoculation method.

TABLE 1.—*Susceptibility of barley varieties to the Missouri Early Beardless collection of Ustilago nuda when inoculated by the partial-vacuum method*

Variety	Tests	Seeds planted	Plants obtained	Plants infected	Average infection[a]
	Number	*Number*	*Number*	*Number*	*Per cent*
Missouri Early Beardless	8	858	754	399	52.92 ± 7.61
Colsess IV	8	563	504	381	75.59 ± 12.39
Trebi	9	649	514	7	1.36 ± 0.607
Hordeum deficiens	5	521	477	5	1.05 ± 0.246

[a] Standard errors.

INFECTION OF HYBRID PROGENIES

In order to expose the progeny of a hybrid barley plant to infection by *Ustilago nuda*, the heads were inoculated at flowering time. The tissues through which the infecting hyphae penetrate to the ovary are those of the parent plant, which may differ genetically from those of the embryo to be tested for susceptibility. It was necessary, therefore, to ascertain whether the results of inoculation were influenced by the resistance or susceptibility of the parent-plant tissue or by the resistance or susceptibility of the em-

bryo. For example, when the flowers of F_1 hybrids were inoculated, was the appearance of smut in the F_2 plants dependent on the ability of the fungus to penetrate the floral tissues of the F_1 hybrid, or on its ability to infect the tissues of the F_2 embryo? An examination of the F_1 hybrids of reciprocal crosses between resistant and susceptible varieties should yield information on this point. The F_1 generation was tested by inoculating the flowers of the female parent 24 hours after the introduction of pollen from the male parent. Unfortunately, F_1 material of only one series of reciprocal crosses was available.

Crosses between Trebi (1.36 per cent susceptible) and Colsess IV (75.59 per cent susceptible), in which Trebi was used as the female parent (Table 2), yielded 57 F_1 plants, of which 3.51 per cent were infected. The reciprocal cross yielded 48 F_1 plants, of which 6.25 per cent were infected. Although the percentage of infection was considerably higher when the susceptible female parent was inoculated, the difference is not significant.

A further comparison of the effect of the reaction of the parent on embryo infection may be drawn from the results of inoculation of the F_1 hybrids, Trebi × Missouri Early Beardless and Missouri Early Beardless × *Hordeum deficiens*. When Trebi (1.36 per cent susceptible) was pollinated with pollen of Missouri Early Beardless (52.92 per cent susceptible) and inoculated, 84 F_1 plants were secured, of which 1.19 per cent were infected. When stigmas of Missouri Early Beardless (52.92 per cent susceptible) were pollinated with pollen of *H. deficiens* (1.05 per cent susceptible) and inoculated, all of the 36 F_1 hybrids showed no infection. While the data are not extensive, there are clear indications that the infection of the hybrids is determined by the nature of the embryo, rather than by the resistance or the susceptibility of the floral tissues of the female parent on which they are borne.

The hypothesis that stigmatic tissues are susceptible to invasion by infection hyphae is further supported by the behavior of the F_2 hybrids. When the stigmas of F_1 hybrids are inoculated, the infection hyphae must penetrate the floral tissues bearing the F_1 complement of genes for resistance and susceptibility. That such tissues are susceptible is proved by the increased percentages of infection among F_2 plants (Table 3).

Similar results were obtained by Larose and Vanderwalle (2) and Milan (3) with wheat hybrids inoculated with *Ustilago tritici* (Pers.) Rostr. They inoculated plants of the F_1 generation of reciprocal crosses and found resistance dominant, regardless of whether a resistant or a susceptible plant was used as the female parent.

INHERITANCE OF RESISTANCE FACTORS

The study of the method of inheritance of resistance to *Ustilago nuda* was complicated by the fact that it was not possible to obtain 100 per cent infection, even in Colsess IV, the most susceptible variety tested (Table 1). Since the breeding program was directed primarily toward incorporating

desirable agronomic characters with smut resistance, many of the crosses involved the use of Missouri Early Beardless, which showed an average infection of about 50 per cent by the most effective inoculation methods available. It was considered desirable, therefore, to observe the behavior of inoculated F_1, F_2, and F_3 progenies.

F_1 Generation

The data for the infection of F_1 barley hybrids inoculated with chlamydospores of *Ustilago nuda* (Table 2) suggest that the resistance of Trebi and *Hordeum deficiens* is transmitted as a dominant character. Since neither variety is immune, an occasional smutted plant is to be expected among the F_1 hybrids. In the reciprocal crosses of Trebi with Colsess IV, however, the percentage of infection of inoculated F_1 hybrids was somewhat greater than that obtained by inoculation of Trebi, indicating that the resistance of Trebi may not be completely dominant.

TABLE 2.—*Infection of F_1 barley hybrids inoculated with chlamydospores of Ustilago nuda*

Hybrid	Total plants	Infected plants	
	Number	*Number*	*Per cent*
Colsess IV × Trebi	48	3	6.25
Trebi × Colsess IV	57	2	3.51
Trebi × Missouri Early Beardless	84	1	1.19
Missouri Early Beardless × *Hordeum deficiens*	36	0	0.0

F_2 Generation

Infection data for this generation are shown in table 3. The proportion of infected plants in the reciprocal crosses of Colsess IV with Trebi suggests a dihybrid ratio. It must be borne in mind, however, that the susceptible parent did not develop 100 per cent infection when inoculations were made at different periods, even though care was taken to maintain uniform conditions for inoculation and incubation. It, therefore, appears unlikely that resistance is dependent on the occurrence of two dominant factors, either of which might confer resistance on the progeny.

When Missouri Early Beardless was crossed with either Trebi or *Hordeum deficiens*, a very low percentage of infection was obtained in the second generation as compared with that obtained when Trebi was crossed with Colsess IV. Since the variety Trebi was used in crosses with both Missouri Early Beardless and Colsess IV, there is an indication that the difference in susceptibility of Missouri Early Beardless and Colsess IV (Table 1) is apparent also in the hybrid progenies from crosses involving those two varieties.

The large percentage of resistant plants in the crosses of Missouri Early Beardless with Trebi and with *Hordeum deficiens* indicates that the inheri-

tance of resistance to *Ustilago nuda* may be controlled by several genetic factors. Since the crosses of Trebi and Colsess IV indicate the probable existence of a single factor for resistance in Trebi, it seems possible that a factor for resistance also may have been introduced into the Trebi × Missouri Early Beardless cross by the Missouri Early Beardless parent. Crosses between *Hordeum deficiens* and Missouri Early Beardless reacted similarly to the Trebi × Missouri Early Beardless cross.

TABLE 3.—*Infection of F_2 barley hybrids inoculated with chlamydospores of Ustilago nuda*

Hybrid	Seeds planted	Plants	Infected plants	
	Number	*Number*	*Number*	*Per cent*
Colsess IV × Trebi	142	112	12	10.71
Trebi × Colsess IV	216	212	16	7.54
Total	358	324	28	8.64
Missouri Early Beardless × *Hordeum deficiens*	109	97	3	3.09
Hordeum deficiens × Missouri Early Beardless	211	186	8	4.30
Total	320	283	11	3.88
Trebi × Missouri Early Beardless	234	191	4	2.09
Colsess IV × Missouri Early Beardless	133	117	69	58.97

In view of the apparent difference in reaction of the hybrid progenies when the two susceptible varieties, Colsess IV and Missouri Early Beardless, were used with the same resistant variety, it was of interest to observe the infection obtained in inoculated F_2 hybrids from the cross, Colsess IV × Missouri Early Beardless. Of a total of 117 inoculated F_2 plants, 58.97 per cent were infected (Table 3). This more nearly approaches the average percentage of infection obtained by inoculating Missouri Early Beardless instead of Colsess IV, and may indicate that Missouri Early Beardless possesses a weak factor for resistance that is absent in Colsess IV.

F_3 Generation

The F_3 progenies were classified as either resistant, segregating, or susceptible. The separation of the segregating and susceptible progenies was more or less arbitrary. Due to variation in effectiveness of inoculation and to the failure to obtain 100 per cent infection of the susceptible parent, it was difficult to separate the segregating and susceptible classes. Since the lowest percentage of infection obtained by inoculation of Colsess IV was 58.63, progenies in which the percentage of infected individuals was more than 55 per cent were arbitrarily classified as susceptible; the segregating progenies included those in which the percentage of infected individuals was 1 to 55; progenies free from infection were considered resistant. The lowest

percentage of infection obtained by inoculation of Missouri Early Beardless with *Ustilago nuda* was 35.90; therefore, 35 per cent was chosen as the separation point between segregating and susceptible progenies when Missouri Early Beardless was used as the susceptible parent. Some erroneous classifications probably were made, particularly among progenies containing small numbers of plants. The average number of plants in the F_3 progenies was 19.3. Progenies containing less than 12 plants were not included.

A total of 122 families inoculated with *Ustilago nuda* were grown from a smut-free F_2 generation of the cross Colsess IV × Trebi (Table 4). There were 56 F_3 families with 887 individuals containing no smutted plants, while 66 families containing 955 plants showed infection ranging from 7.69 to 100 per cent. Only 6 of these families had less than 25 per cent infection, while 4 had 100 per cent. A total of 31 families (25.41 per cent) contained over 55 per cent of infected plants, which is within the limits of susceptibility of the susceptible parent. The greater number of infected F_3 populations was fairly well distributed between 20 and 75 per cent, with some tendency toward the formation of two groups, one between 20 and 40 per cent, and the other between 50 and 75 per cent.

TABLE 4.—*Infection of F_3 families from non-inoculated plants when inoculated with chlamydospores of Ustilago nuda*

Hybrid	Prog.	Seeds pltd.	F_3 plants	F_3 families in infection percentage classes		
				0.0	1–54.99	55–100
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Colsess IV × Trebi	122	2357	1842	56	35	31
Trebi × Colsess IV	62	1150	939	22	21	19
Total	184	3507	2781	78	56	50

The reciprocal cross, Trebi × Colsess IV, reacted similarly (Table 4). The number of susceptible progenies in both crosses approaches that expected if resistance is controlled by a single genetic factor. When the data from the reciprocal crosses are combined, 184 F_3 progenies are distributed in 5 per cent groups (Fig. 1). The percentage of families that reacted in a manner characteristic of the susceptible parent suggests that the inheritance of resistance is dependent on a single factor. The preponderance of resistant progenies and the small number of segregating progenies may be due to the small numbers of plants in the families, or to variation in effectiveness of the inoculation methods, and failure to secure 100 per cent infection in the susceptible parent, or, yet again, to partial failure of germination. Thren (9) reported that introducing a large number of viable spores into a flower reduced its ability to produce viable seed. He found that the loss, 21.4 per cent of the inoculated seeds as compared with 7 per cent of the con-

trols, was due primarily to failure of the seeds to germinate, or, to weak germination followed by death of the seedlings.

Like results were obtained in this investigation. A larger percentage

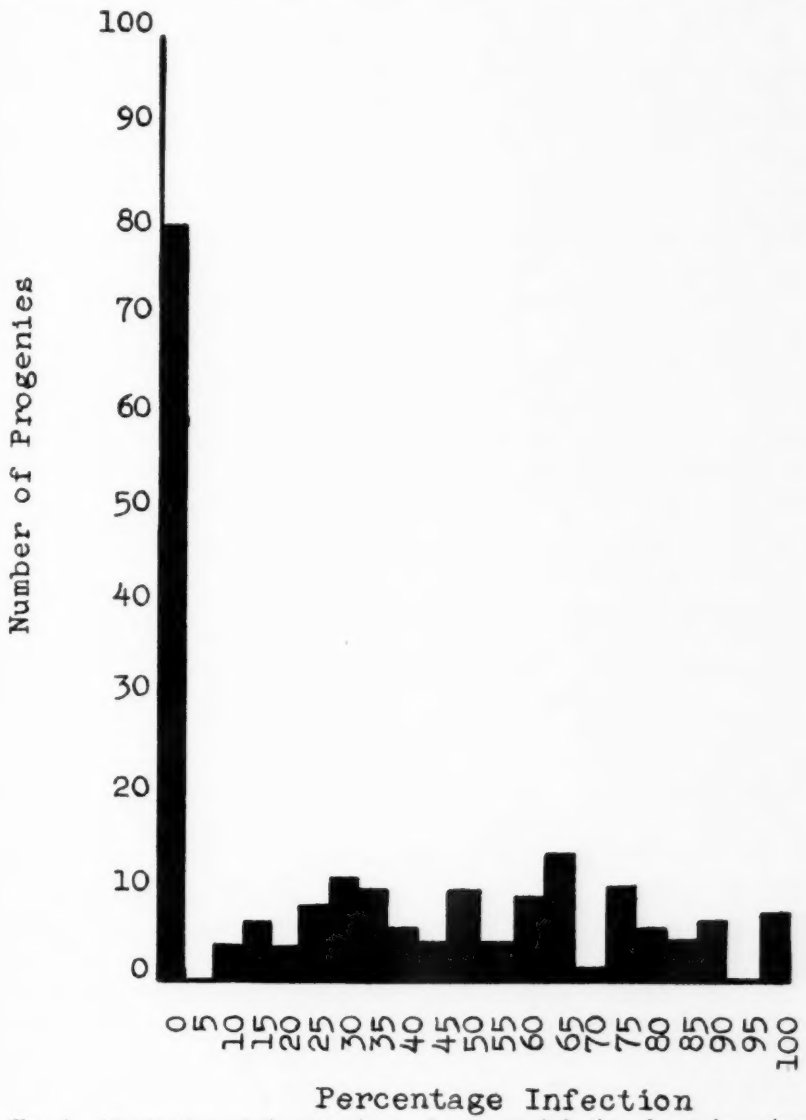

FIG. 1. Distribution of F_3 progenies in 5 per cent infection classes in reciprocal crosses of Trebi with Colsess IV when artificially inoculated with chlamydospores of *Ustilago nuda*.

of seeds in the F_3 families containing infected plants failed to germinate than in F_3 families with no infection (Table 5), indicating that the penetration of the embryo of the barley flower by the mycelium of *Ustilago nuda* has a detrimental effect on the development and maturation of the seed,

TABLE 5.—*Percentages of non-viable seeds in infected and uninfected F_3 families following inoculation with chlamydospores of Ustilago nuda*

Hybrid	Seeds planted		F_3 plts. obtained		Non-viable seeds	
	Non-inf. prog.	Inf. prog.	Non-inf. prog.	Inf. prog.	Non-inf. prog.	Inf. prog.
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
Colsess IV × Trebi	1052	1305	887	955	15.69	26.82
Trebi × Colsess IV	385	772	327	612	15.07	20.72
Trebi × Missouri Early Beardless	2999	1861	2686	1619	10.44	13.01
Missouri Early Beardless × *Hordeum deficiens*	752	467	660	384	12.24	17.78
Hordeum deficiens × Missouri Early Beardless	927	594	793	468	14.46	21.22

resulting either in failure of the seed to germinate, or in the production of a weak seedling. This tends to eliminate susceptible plants in the progenies and to cause some of the segregating progenies to be classified as resistant.

TABLE 6.—*Infection of F_3 families from non-inoculated plants when inoculated with chlamydospores of Ustilago nuda*

Hybrid	Prog.	Seeds pltd.	F_3 plants	F_3 families in infection percentage classes		
				0.0	1–34.99	35–100
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Trebi × Missouri Early Beardless	172	4860	4305	104	43	25
Missouri Early Beardless × *Hordeum deficiens*	72	1212	1044	47	10	15
Hordeum deficiens × Missouri Early Beardless	96	1472	1261	61	18	17

The infection data of the hybrids from Trebi with Missouri Early Beardless, and *Hordeum deficiens* with Missouri Early Beardless are presented in table 6. The distribution of families in 5 per cent infection groups is shown in figure 2. They do not harmonize with the 1:2:1 ratio because of a preponderance of resistant progenies. The average percentage of infection in segregating and susceptible F_3 progenies of the Trebi × Missouri Early Beardless cross was also low (36.53) when compared with 52.86 per cent infection of comparable F_3 progenies from the cross, Colsess IV × Trebi. These results indicate the possible existence of a factor for resistance in the hybrids from Trebi × Missouri Early Beardless, which was not present in the Colsess IV × Trebi hybrids.

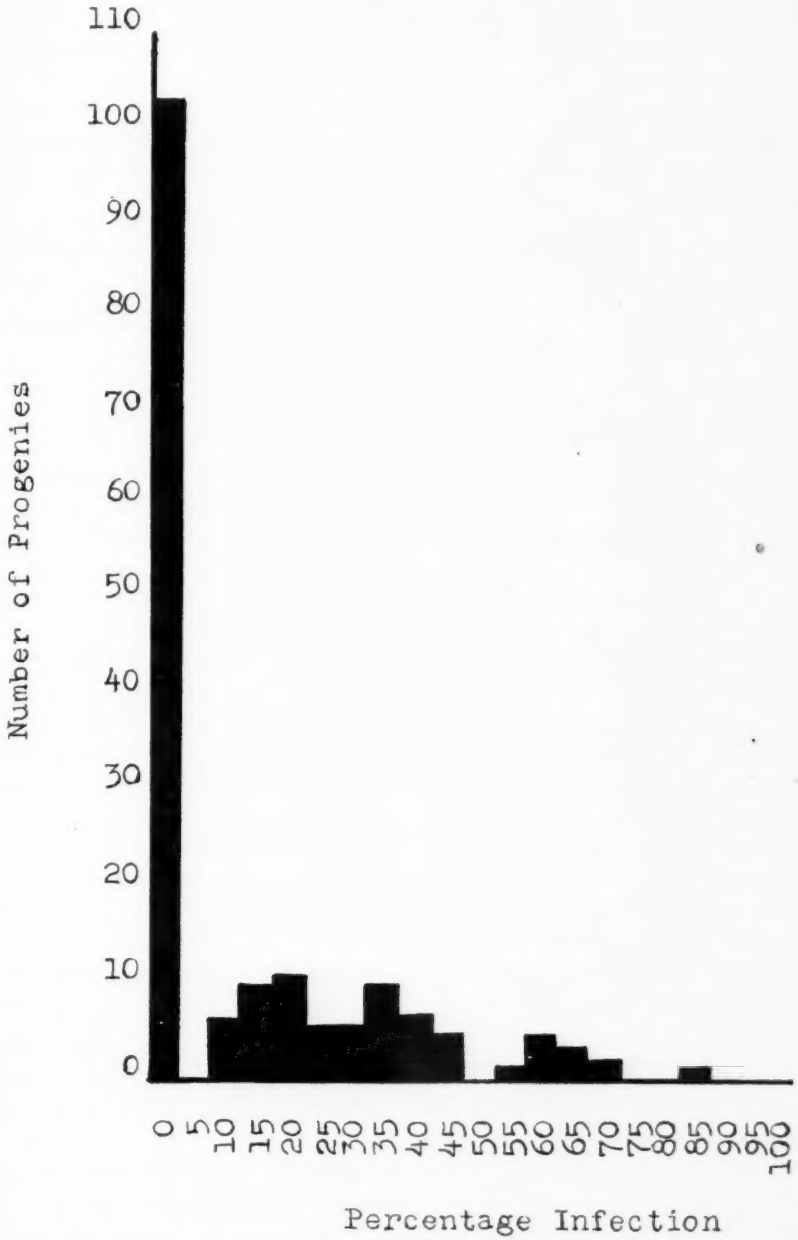

FIG. 2. Distribution of F_3 progenies in 5 per cent infection classes in the cross of Trebi × Missouri Early Beardless when inoculated with chlamydospores of *Ustilago nuda*.

The average percentages of infection of the segregating and susceptible F_3 progenies obtained when Missouri Early Beardless was crossed with *Hordeum deficiens* were nearly the same as that obtained when Missouri Early Beardless was crossed with Trebi (Table 7). The hybrids from each

cross also reacted similarly with respect to the numbers of resistant, segregating, and susceptible progenies. *H. deficiens* probably possesses a single factor for resistance, which is similar in its effect to the factor carried by Trebi. The large number of resistant progenies may be due to a weak factor for resistance in Missouri Early Beardless. The existence of such a factor was indicated, also, by the behavior of Trebi × Missouri Early Beardless hybrids.

TABLE 7.—*Percentages of infection by Ustilago nuda in inoculated segregating and susceptible F_3 progenies of various barley hybrids*

Hybrid	Families	Plants	Infected plants	
	Number	*Number*	*Number*	*Per cent*
Trebi × Missouri Early Beardless	68	1619	517	31.93
Missouri Early Beardless × *Hordeum deficiens*	25	384	155	40.36
Hordeum deficiens × Missouri Early Beardless	35	468	171	36.53
Colsess IV × Trebi	62	1139	602	52.86

Backcross Progenies

The F_2 plants from the backcross were inoculated by introducing the spores of *Ustilago nuda* into the flowers of the F_1 plants. The data are presented in table 8. Of 98 inoculated F_2 families, obtained by backcrossing F_1 hybrids of Trebi × Colsess IV onto Colsess IV, 21 were free from infection, while 77 contained 1 or more infected individuals. If resistance is controlled by a single factor, all of the F_2 families from the backcross should show some infection with a tendency for the progenies to accumulate in two infection groups. If 25 per cent is arbitrarily taken as the upper limit of susceptibility of the progenies from heterozygous backcross-generation plants, 57 progenies would be classified as heterozygous and 41 as homozygous for susceptibility. These figures agree reasonably well with the expected segregation of 1 heterozygous : 1 homozygous progeny, in view of the afore-mentioned difficulties in obtaining infection of all susceptible plants.

Of the F_2 plants, obtained by backcrossing F_1 hybrids of Trebi × Missouri Early Beardless onto Missouri Early Beardless, there were 61 progenies showing less than 25 per cent infection, and only 23 progenies showing 25 per cent or more infection. This segregation does not conform to the 1 heterozygous : 1 homozygous ratio expected when a single factor for resistance is operating. The resistant parent used in this backcross was the same as that used in producing the hybrids of the backcross, Colsess IV × (Trebi × Colsess IV), which segregated reasonably close to the 1 : 1 ratio when inoculated under similar conditions. This further indicates that the Missouri Early Beardless parent probably contributes a factor for resistance that tends to reduce the susceptibility of the hybrids, in addition to the factor for resistance contributed by Trebi.

TABLE 8.—*Infection of the first-selfed generation progenies from the backcross when inoculated with chlamydospores of Ustilago nuda*

Backcross	Prog.	Plants	Progenies in infection percentage classes		
			0.0	1–24.99	25–100
	Number	*Number*	*Number*	*Number*	*Number*
Colsess IV × (Trebi × Colsess IV)	98	1766	21	36	41
Missouri Early Beardless × (Trebi × Missouri Early Beardless)	84	1219	32	29	23

ASSOCIATION OF CHARACTERS

Hybrids from the cross, Missouri Early Beardless × *Hordeum deficiens*, were used for studying the association between morphological characters. They were particularly desirable for a study of the association, since the parents differed in the expression of 3 morphological characters, important from the standpoint of development of a desirable variety of winter barley. Missouri Early Beardless possessed the 3 desirable characters, hoods, six-row heads, and winter habit, but was susceptible to *Ustilago nuda*, whereas *H. deficiens* possessed awns, *deficiens*-type heads, spring habit, and resistance to *Ustilago nuda*.

The factor pairs for the hooded *vs.* awned, and the non-six-row *vs.* six-row condition have been placed in separate linkage groups (6) and confirmed in this investigation. No reports have been found in the literature concerning linkage relations of the factors that control spring *vs.* winter habit in barley.

Segregation and linkages in the second generation were determined by observing the segregation of uninoculated F_2 progenies.

Spring *vs.* Winter Habit

F_1 Generation. All of the 36 F_1 plants of the cross, Missouri Early Beardless × *Hordeum deficiens*, showed the spring habit of growth, indicating dominance of that habit. This was true also of the first generation plants from the cross of Trebi × Missouri Early Beardless.

TABLE 9.—*F_2 segregation of spring vs. winter habit in barley hybrids from Missouri Early Beardless × Hordeum deficiens and reciprocal*

	Spring	Winter	χ^2	P
	Number	*Number*		
Obs.	161	40	2.7876	0.50
Theor. 3:1	150.75	50.25		

F_2 Generation. The progenies from 201 selfed F_1 plants were observed and the results recorded in table 9. One hundred sixty-one of the F_2 plants

were classified as spring type and 40 as winter type. When compared with the 3 : 1 ratio expected when the segregation is controlled by a single genetic factor, chi-square was 2.7876. This is a satisfactory agreement between the observed and the calculated frequencies, and indicates that a single factor pair controls the inheritance of spring and winter habit, with the factor for spring habit dominant.

Hoods *vs.* Awns with Spring *vs.* Winter Habit

F_2 segregation of hybrids from the cross, Missouri Early Beardless × *Hordeum deficiens*, indicates that the two pairs of factors for hoods *vs.* awns and spring *vs.* winter habit are inherited independently (Table 10). When compared with the normal 9 : 3 : 3 : 1 ratio, chi-square was 0.5975, indicating that the factor pairs determining the above characters are not in the same linkage group.

TABLE 10.—*F_2 association of morphological characters in Missouri Early Beardless × Hordeum deficiens hybrids*

Plant type	Spring		Winter		χ^2	P
	Obs.	Theor. 9 : 3 : 3 : 1	Obs.	Theor. 9 : 3 : 3 : 1		
	Number	*Number*	*Number*	*Number*		
Hooded × awned						
Hooded	63	65.25	24	21.75	0.5975	> .8
Awned	23	21.75	6	7.25		
Non-six-row × six-row						
Non-six-row	66	65.25	25	21.75	1.3332	> .7
Six-row	20	21.75	5	7.25		

Non-six-row *vs.* Six-row with Spring *vs.* Winter Habit

When the frequencies obtained in the segregation of non-six-row *vs.* six-row with spring *vs.* winter habit were compared with the normal 9 : 3 : 3 : 1 ratio expected when 2 factor pairs segregate independently (Table 10), chi-square was 1.3332. This is not a significant deviation from the values expected with independent segregation.

It may be concluded from the data presented on the segregation of morphological characters that the 3 factor pairs determining hoods *vs.* awns, non-six-row *vs.* six-row heads, and spring *vs.* winter habit are located in separate linkage groups.

Resistance *vs.* Susceptibility with Hoods *vs.* Awns

The data for the comparison of the frequencies of resistant and susceptible F_2 progenies with hooded and awned progenies are presented in table 11. Because of the difficulty of accurately classifying the resistant, segregating, and susceptible progenies, a theoretical ratio, with which linkage intensities may be compared, cannot be formulated. In an attempt,

however, to obtain some indication of possible linkage, the data are compared with frequencies expected when segregation is controlled by 2 independent factor pairs.

TABLE 11.—*F_2 segregation of hoods vs. awns with resistance vs. susceptibility*

Cross	Ratio	Hoods		Awns		Total
		Resis.	Suscep.	Resis.	Suscep.	
		Number	*Number*	*Number*	*Number*	*Number*
Colsess IV × Trebi and reciprocal	Obs.	104	38	30	12	184
	Theor. 9:3:3:1	103.50	34.50	34.50	11.50	
Missouri Early Beardless × *Hordeum deficiens* and reciprocal	Obs.	110	23	26	9	168
	Theor. 9:3:3:1	94.50	31.50	31.50	10.50	
Trebi × Missouri Early Beardless	Obs.	111	22	36	3	172
	Theor. 9:3:3:1	96.75	32.25	32.25	10.75	

There was no indication of linkage between the factor pairs determining resistance *vs.* susceptibility and hoods *vs.* awns in hybrids from Colsess IV × Trebi. Segregation for resistance in hybrids from this cross was apparently controlled by a single factor, and when the data are compared with the segregation for hoods *vs.* awns, it approaches the 9:3:3:1 ratio expected with independent segregation of 2 factor pairs.

The data for the segregation of the factor pairs in hybrids from Missouri Early Beardless with *Hordeum deficiens* and Trebi do not agree with the 9:3:3:1 ratio. There were more resistant plants and fewer susceptible ones in both the hooded and awned classes than was to be expected with segregation of two independent factor pairs. The segregation of the hybrids for resistance and susceptibility indicated the presence of a single dominant factor for resistance supplied by the resistant parents and an additional weak factor for resistance furnished by the Missouri Early Beardless parent. The possible presence of this second factor and the difficulty of obtaining universal infection of the susceptible progenies probably accounts for the large number of resistant progenies in comparison to the number of susceptible ones. For these reasons it is doubtful if there is any linkage between the factor pairs determining resistance *vs.* susceptibility and hoods *vs.* awns in any of the crosses studied.

Resistance *vs.* Susceptibility with Non-six-row *vs.* Six-row

There was no indication of linkage between the factors determining resistance and susceptibility and those determining non-six-row and six-row heads. When the data are arranged in 4 classes, the numbers approach those expected on the basis of independent segregation (Table 12).

TABLE 12.—*F_2 association of characters in reciprocal crosses of Missouri Early Beardless × Hordeum deficiens*

Plant type	Resistant		Susceptible		Total
	Obs.	Theor. 9 : 3 : 3 : 1	Obs.	Theor. 9 : 3 : 3 : 1	
	Number	*Number*	*Number*	*Number*	
Non-six-row × six-row					
Non-six-row	96	95.04	21	31.68	
Six-row	40	31.68	11	10.56	168
Spring × winter					
Spring	53	47.81	20	15.93	
Winter	3	15.93	9	5.31	85

Resistance *vs.* Susceptibility with Spring *vs.* Winter Habit

The data for the segregation of the factor pairs determining resistance *vs.* susceptibility and spring *vs.* winter habit are presented in table 12.

Of the 85 inoculated F_2 plants only 3 possessed resistance and winter habit. This is a much smaller number of resistant winter plants than was to be expected, since resistance is transmitted as a dominant character. There were more susceptible winter-type than resistant winter-type individuals, suggesting the possibility of linkage between the factors for winter habit and susceptibility; however, the number of plants is not sufficiently large to permit definite conclusions.

In the field there would probably be less evidence of linkage between the factors for winter habit and susceptibility because infected embryos often produce weak plants, which would be eliminated by winter conditions. Thren (9) reported weak germination of seeds from flowers inoculated with a large number of viable spores of *Ustilago nuda;* the loss was higher in winter than in spring barley, the result of the inability of weak, infected plants to withstand adverse weather conditions.

DISCUSSION

The exact nature of the inheritance of resistance in barley to *Ustilago nuda* was not determined because of the failure to identify all susceptible and segregating genotypes. An inoculation method that will give 100 per cent infection with *U. nuda* has not yet been devised. Another complicating factor is the possibility of the presence of several physiologic forms of *U. nuda* in the inoculum, even though the inoculum supply were built up from a single infected head of Missouri Early Beardless barley by repeated inoculation of that variety. A third factor, previously pointed out by Roemer (7) and Thren (9), is that infected plants may be weakened by the presence of the loose-smut fungus, which results in a selective death of seedlings. This difficulty was partly eliminated in the present investigation by germinating the seeds in the laboratory, planting in plant bands, and growing to a height of 3 inches in a cold frame before transplanting to the

field. Progenies that survived the winter in the field were, however, undoubtedly subject to loss of infected seedlings, possibly accounting, in part, for the preponderance of resistant plants and progenies.

It was apparent, however, that resistance to *Ustilago nuda* was inherited as a dominant character. The F_1 generation usually equalled or approached the resistance of the resistant parents, although in crosses between Trebi and Colsess IV there was some evidence that dominance may not be complete. In later generations there was apparently an excess of resistant plants. Nahmmacher (5) and Zeiner (10) found resistance to *U. nuda* was completely dominant and controlled by a single factor in certain barley crosses.

Resistance appeared to be physiological. When flowers were inoculated on a plant carrying factors for resistance in a heterozygous condition, both resistant and susceptible plants were obtained. Hyphae from the fungus spores must reach the ovule in order to infect the susceptible embryos. Except for the tissues that developed following fertilization of the egg nucleus within the embryo sac, the tissues of the flowers that produced resistant and susceptible plants were the same. Therefore, the hyphae must be able to penetrate the floral tissues to the embryo in plants carrying factors for resistance in the dominant condition. The infection of the F_1 plants of reciprocal crosses (Table 2) also suggests that the fungus reaches the embryos with approximately equal frequency, whether the female parent is resistant or susceptible. Larose and Vanderwalle (2) and Milan (3) found this true of the infection of F_1 progenies of reciprocal crosses between very susceptible and very resistant wheat varieties inoculated with *Ustilago tritici*. The resistance of the F_1 plants approached that of the resistant parent, regardless of the resistance of the female parent.

SUMMARY

The inheritance of resistance to the brown loose smut of barley, caused by *Ustilago nuda*, was studied in barley hybrids. The two susceptible varieties, Missouri Early Beardless and Colsess IV, and the resistant varieties Trebi and *Hordeum deficiens* were used as parents.

Infection reactions of F_1 plants from reciprocal crosses of Trebi with Colsess IV indicated that the infection tubes from the chlamydospores of *Ustilago nuda* reached the hybrid embryos with approximately equal frequency, regardless of the resistance or susceptibility of the female parent.

Both Trebi and *Hordeum deficiens* apparently possess a dominant factor for resistance; but dominance may not always be complete. F_2 progenies from crosses of each variety with Missouri Early Beardless were infected to a similar degree, indicating that the resistance factors carried by the 2 resistant parents were similar in their effects.

In F_2 and subsequent generations there was a preponderance of resistant plants, possibly attributable to lethal effects of infection, to variations in percentages of infection obtainable by the methods used, and to failure to obtain universal infection of the susceptible parent.

The susceptibility of F_2 hybrids from a Colsess IV × Missouri Early Beardless cross approached the infection limits of Missouri Early Beardless but was less than that of Colsess IV, suggesting the occurrence of a weak resistance factor in Missouri Early Beardless.

The susceptibility of first-selfed backcross generation progenies was in agreement with the susceptibility of F_2 and subsequent generations, offering additional evidence for the presence of a single dominant factor for resistance in Trebi and *Hordeum deficiens*, and possibly a weak factor for resistance in Missouri Early Beardless.

There was no evidence of linkage between the factors for resistance and those for hoods or six-rowness.

Promising selections of hooded, six-row, winter barleys resistant to *Ustilago nuda* were obtained.

NEBRASKA AGRICULTURAL EXPERIMENT STATION,
LINCOLN, NEBRASKA.

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A METHOD FOR THE CULTURE OF SEEDLINGS AND SMALL PLANTS IN SUNLIGHT UNDER CONTROLLED TEMPERATURE CONDITIONS[1]

J. A. Pinckard and Luben Spasoff Bozovaisky[2]

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INTRODUCTION

The effect of ever-changing environment upon growing plants is a perennial problem confronting nearly all botanical investigators. To circumvent the problem numerous techniques have been adopted for stabilizing one or more environmental factors, or to avoid them completely, depending upon the nature of the problem, the facilities available, and the degree of accuracy required. Apparatus of many sorts have been described, but most of them represent an accumulation of expensive equipment requiring shop tools and the services of specialized mechanics and skilled craftsmen for construction and maintenance. Many small laboratories with limited funds and personnel, cannot afford the expenditure of more than a few hundred dollars over a period of years for temperature-control equipment.

The purpose of this paper is to describe a refrigerated plant-culture chamber designed to meet the needs of small-scale greenhouse research. By taking advantage of local commercial refrigeration sales and service the cost may be held to a minimum without sacrificing accuracy and efficiency.

PRINCIPLES OF DESIGN

Since sunlight, passing through glass, seemed to be the most practical means of illumination for growing plants in controlled environments, the chambers were designed for installation in a section of greenhouse with thermostatically controlled heat (Fig. 1). On a bright summer day, when greenhouse temperatures approximate 100° F., without roof shade, the inside cabinet temperature of an unrefrigerated empty chamber may reach 150° F. If a cloud passes between the sun and the chamber, the temperature in a well-ventilated greenhouse may fall several degrees but that of the unrefrigerated chamber may remain exceedingly high depending upon the efficiency of its insulation. Heat of radiation resulting from the sun's rays remains trapped within the insulated chamber, since little of it is conducted through well-insulated walls. This accumulated heat is of little importance, since it arises from surfaces heated by light rays. If surfaces within the chamber are reduced to a minimum and if these are leaf surfaces, as they should be, for optimum efficiency, the rate of radiation, convection, and

[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist and Senior Agricultural Scientific Aide, respectively, Virginia Agricultural Experiment Station. The writers also wish to express their gratitude to F. A. Wolf, W. A. Whitney and others for assisting in many ways.

accumulation of heat in a small plant-growing chamber is not too great to be dissipated by ordinary refrigeration equipment.

If air be passed across these leaf surfaces at low velocity, convected heat of radiation is easily swept away where it may be cooled. Thus, if the design of a plant-growing chamber be such that a maximum working surface of leaf area is exposed to sunlight, together with a minimum surface of heat-absorbing structural materials, low-velocity air circulation may be employed with a minimum of refrigeration equipment. By reducing the non-working

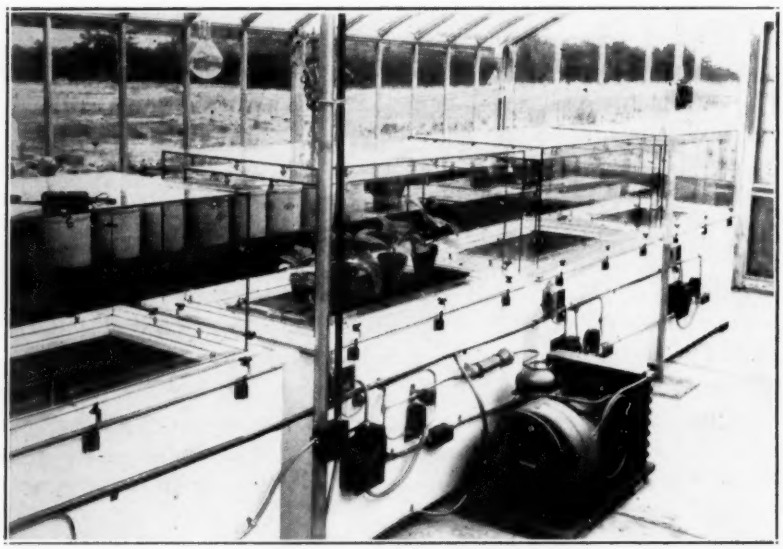

FIG. 1. A battery of 4 plant-culture chambers installed in the experimental greenhouse. Artificial illumination supplemented sunlight when necessary. The compressor assembly, dehydrator, shut-off valve, and solenoid stop valve, all in the small "liquid" line, are shown in the foreground. (See Fig. 2.)

heat-absorbing surfaces (exposed structural materials) in a chamber one may obtain also a better balance between chamber-air temperature and cooling-coil temperature, which facilitates more accurate control.

The basic design for the chambers described in this paper, therefore, consisted of exposing plants to sunlight under glass and sweeping radiated heat away by low velocity air movement. The apparatus used and the results obtained are described below.

MATERIALS AND METHODS

Each insulated cabinet (I. C., Fig. 2) was constructed in two parts, the lower of wood, with cork insulation between the walls, and the upper of glass plates separated by an air space. The lower part of each cabinet (Figs. 2, 3) was provided with a refrigerator door closing against two rubber gaskets. The interior of each box was lined with galvanized iron, soldered at the seams. A small drain pipe, soldered to the lining and extending

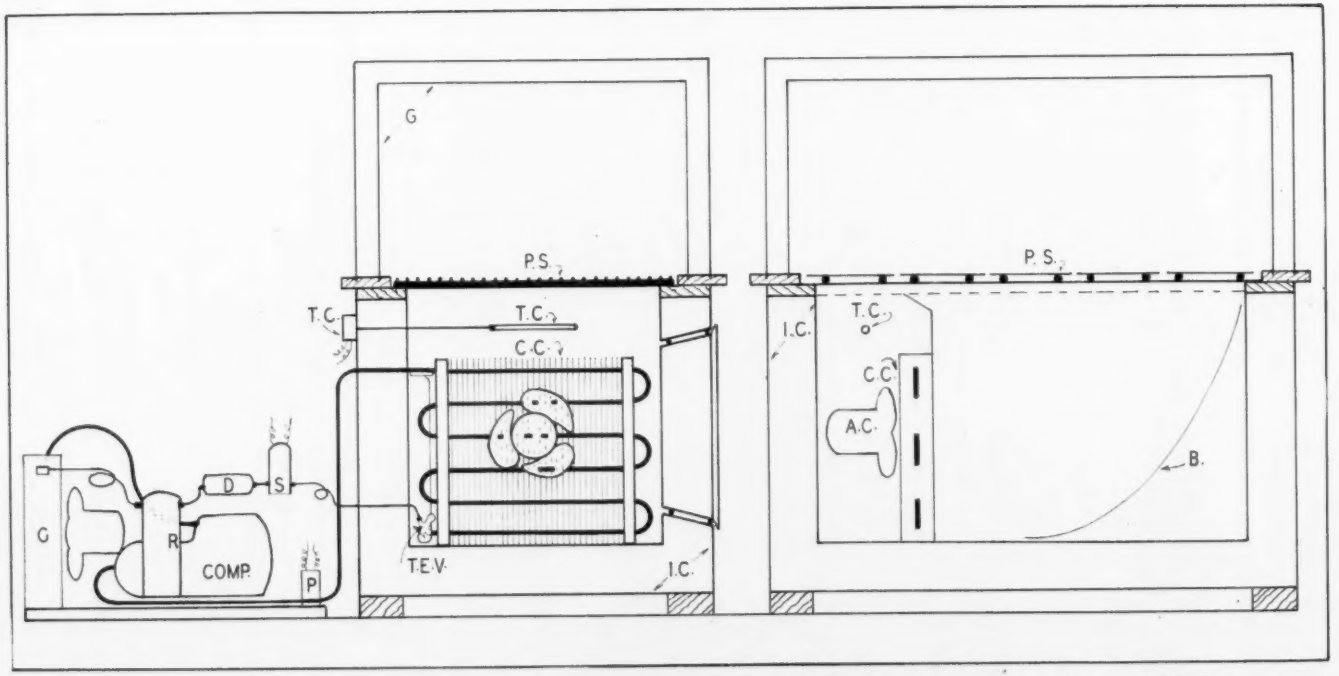

FIG. 2. Diagram of apparatus employed for the culture of seedlings and small plants in sunlight under controlled temperature conditions. A. C., air circulator; B, baffle; C, condenser; C. C., cooling coil; Comp., compressor; D, dehydrator in "liquid" line (positive pressure); G, glass; I. C., insulated cabinet; P, pressure switch in "suction" line (negative pressure); P. S., plant supports; T. C., temperature controller; T. E. V., thermostatic expansion valve.

through the bottom, facilitated cleaning. It also relieved interior gas pressures caused by temperature changes or by opening or closing the door. Several hinged wing bolts, attached to the top of the box (Fig. 3), permitted secure fastening of the glass section. A rubber gasket between the box and the glass section insured a leak-proof union.

The glass portion of the chambers was seated in rubber-filled channels in a wood frame. The plates of glass were cemented at the edges with waterproof show-case cement of suitable quality. Show-case clamps were used to hold the glass in place. The above method was adopted to minimize shading, which other more bulky structural materials would cause if used to hold the

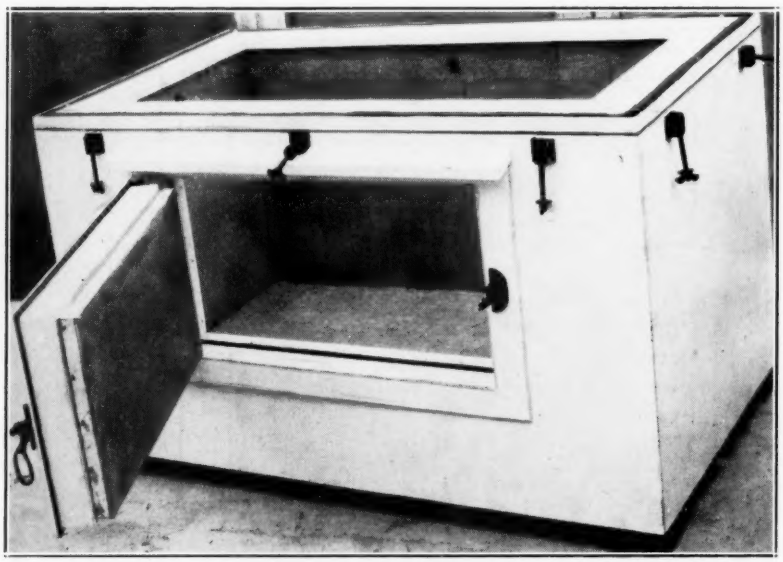

FIG. 3. Lower portion of insulated cabinet (I. C.) consisting of a metal-lined, cork-insulated, wooden box with refrigerator type door.

glass in place. Suitably placed holes in the wood frame supporting the glass permitted escape of air entrapped between the two glass walls. Tubes of a suitable drying agent connected to these small openings prevented moisture from collecting on the glass between the walls in case a leak in the glass union should develop.

The outside dimensions of the cabinets herein described were approximately 48 in. long by 33 in. wide by 51 in. high. An unobstructed surface approximately 38 by 36 in. was available for plants growing in flats. By rearranging the plant supports (P. S., Fig. 2), potted plants up to 36 in. high could be introduced, though with some inconvenience.

Iron grids, made in small sections for easy removal, were used to support the flats of soil or potted plants. The plants were introduced through the door of the chamber and raised into position on the grid. Space at each end of the chamber was kept clear to allow free circulation of air against the

chamber walls. The success of this type of chamber depends upon the movement of large volumes of air at low velocity across all interior surfaces.

The unassembled apparatus, used for controlling temperatures within each chamber, is shown in figure 4. A diagram of the assembled apparatus,

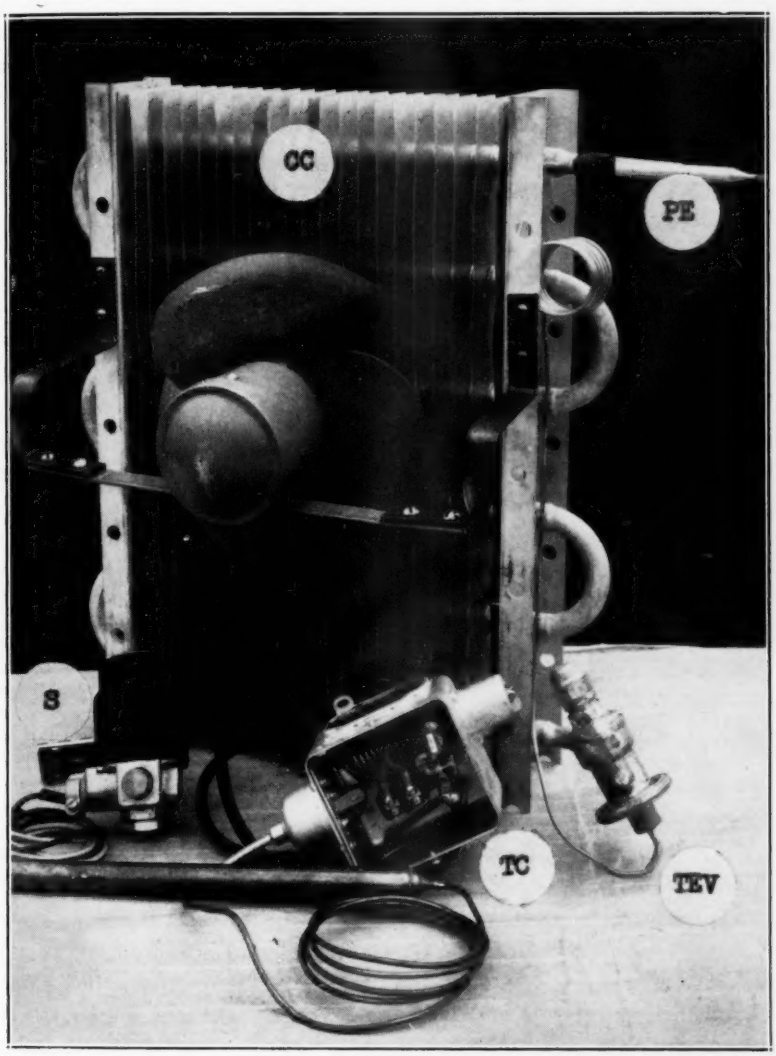

FIG. 4. Refrigeration apparatus required for one plant chamber. C. C., cooling coil; T. E. V. thermostatic expansion valve with its power element; T. C., temperature controller; S, solenoid stop valve.

connected to the compressor unit, is shown in figure 2. A description of each unit of the apparatus follows:

The condensing unit,[3] or compressor assembly (cf. Fig. 2), used to oper-

[3] Model AFE–50, manufactured by Westinghouse Electric Company.

ate all 4 chambers consisted of a compressor and a one-half horsepower motor hermetically sealed. The refrigerant employed was "Freon-12" (difluorodichloromethane, CF_2Cl_2). If the average refrigerant temperature used is 40° F. and if the surrounding air temperature is 100° F., the above unit, if operated continuously, was rated at 7120 BTU an hour. This rating is equivalent to 1186 pounds of ice a day.

Upon condensing the gaseous refrigerant in the condenser (C., Fig. 2) it was collected in receiver R, and held under pressure by means of suitable automatic values at S, and one within the compressor itself. A vacuum was thus established at valve P.

A rise in temperature within the chamber caused the temperature controller bulb T. C. to close an electrical circuit that opened the solenoid stop valve S. Liquid refrigerant then passed from receiver R, through dehydrator D, into the thermostatic expansion valve T. E. V. This valve, operated by means of its power element, attached to the upper tube of the cooling coil, permitted liquid refrigerant to enter the bottom of cooling coil C. C. until the lowest temperature of the cooling coil reached a predetermined value. For ordinary work this valve was adjusted to close at a temperature above freezing, thus preventing frost from collecting on the coil and subsequent dehydration of the chamber air. By carefully adjusting the thermostatic expansion valve T. E. V., when the chamber was in normal operation, sudden temperature changes were avoided, since the temperature of the coil could be regulated to slightly below that required for the chamber. Under ideal conditions this adjustment should operate the cooling coil at a temperature sufficiently below that of the chamber to compensate for heat loss through the chamber walls. If very low temperatures are required, the temperature difference between the coil and the chamber must be increased. The thermal stability of a well-insulated chamber, the door of which is opened infrequently, is scarcely affected by external room temperatures when the coil temperature is balanced with the chamber heat loss.

As refrigerant enters the lower part of the cooling coil it expands into a gas and cools the coil. As pressure is increased in the cooling coil, valve P is caused to close an electrical circuit which starts the compressor and reestablishes positive pressure at point S and negative pressure at P.

A slow-speed air circulator A. C., operated by an induction motor, caused low-velocity air movement across the various surfaces within the chamber.

Humidifying and dehumidifying equipment of various sorts was used by placing the apparatus in the air stream on the bottom of the chamber between the cooling coil and the rubber curtain baffle B.

With the greenhouse temperature thermostat adjusted at 75° F., refrigeration was required in all chambers operating below this level, while heat was usually required above this level at night and on cool, cloudy days. During periods of bright sunshine, and regardless of the greenhouse temperature, refrigeration was usually required in all chambers. By means of an auxiliary heater one chamber was operated very successfully at 105° F.

Refrigeration was required in this chamber during part of the mid-day if bright sunshine prevailed.

The cost of a single cabinet with all equipment was approximately $200. The cost of the compressor assembly complete, was approximately $180. Power consumption during continuous operation was approximately 900 watts an hour.

RESULTS AND DISCUSSION

Although the chambers described in this paper were exposed to sunlight as much as possible in a warm greenhouse, temperatures of the air and soil were held to within approximately 2° or 3° F. of a fixed value. The range of temperature over which these chambers were tested in full sunlight lay between 65° and 105° F. Somewhat higher or lower temperatures could

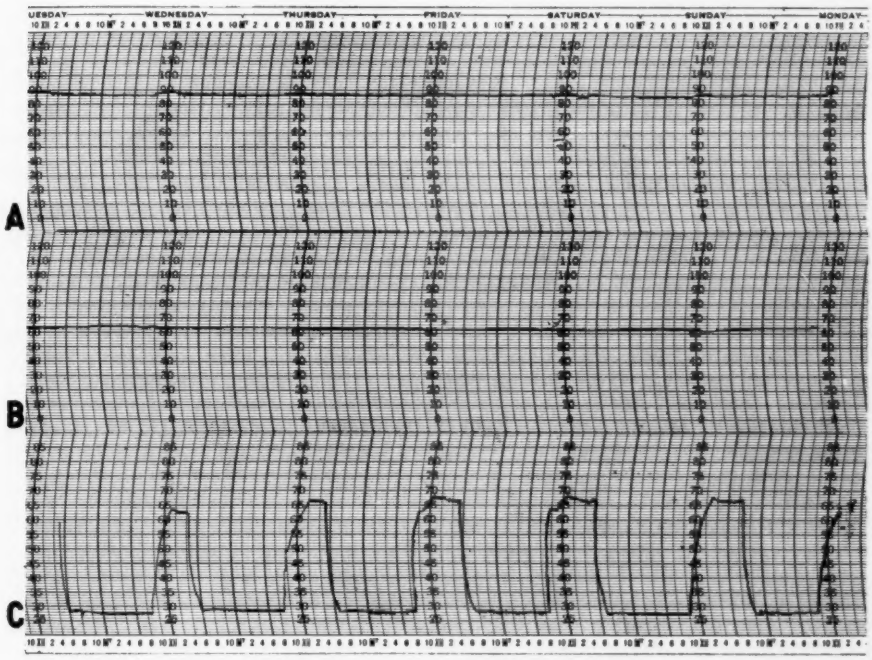

FIG. 5. Thermograph records of the sub-surface soil temperatures. A, 85° to 87° F. chamber; B, 65° to 67° F. chamber; C, 27° to 29° F. chamber. The latter was adjusted nightly for a 14- to 15-hour low-temperature treatment.

undoubtedly have been reached. Figure 5, A and B, shows the thermograph record of two chambers operating under the above-described conditions. A third chamber, C, was adjusted to operate for a 14-hour period during the night at a temperature of between 25° and 27° F. In this chamber no attempt was made to regulate the upper (daytime) temperature except to prevent it from rising above 70° F. All three chambers were in operation continuously over a period of many weeks. No attempt was made to regulate the greenhouse temperatures in which the chambers were located except to maintain a minimum of 75° F. and to provide ventilation on warm days.

Air temperatures, within the chambers, varied, depending upon the opening and closing of the temperature-controller switch. During periods of bright sunshine and intense radiation, the air temperature at a fully illuminated soil surface was frequently as much as 2 or 3 degrees below the sub-surface soil temperature. During periods of cloudy weather or when none of the sun's rays reached the soil, air temperatures were slightly above those of the sub-surface soil layers. Excluding the rate of heat conduction through dry soils, it appears probable that heat exchange, between the soil surface and the air stream immediately above, is affected significantly by surface soil moisture and the amount of moisture in the air blanket immediately above. The use of an appropriate soil cover, such as ground cork, may prove useful in obtaining closer agreement between soil and air temperatures in this of type chamber.

Growth of tobacco seedlings under three thicknesses of glass (two in the chambers and one in the greenhouse) did not seem to be as good as check plants grown on the greenhouse bench. While allowances were made for temperature variation, the quality of the foliage was noticeably different. By providing a longer day for the plants in the chambers, these differences were no longer apparent. The response of Burley and Turkish tobacco seedlings to the temperatures shown in figure 5, A and B, for a period of two weeks[4] is shown in figure 6. The soils in which these seedlings were growing was infested with the black root-rot fungus, *Thielaviopsis basicola* (Berk.) Ferraris. One "acid" soil (pH 5.1) and one "neutral" soil (pH 7.3) was used for each tobacco variety grown at each temperature. This illustration shows the interesting relation between varietal susceptibility, and soil acidity, in a controlled environment. The Burley variety, Judy's Pride, grew very poorly in the acid soil at 65° F., while the Turkish variety, Xanthi, made somewhat better growth. Similar relative growth was made by the two varieties at 85° F., although the Burley variety was more sensitive to the acid soil than was the Turkish variety. In neutral soil at 65° F., the Burley variety was attacked by the root-rot fungus and plant growth was reduced. The Turkish variety, being nearly immune from black root rot, made normal growth. At 85° F., the black root-rot pathogen was not a retarding factor of growth for either variety, although the retarding effect of soil acidity was quite apparent.

During the construction and operation of these chambers the following improvements were suggested that seem worthy of recording: 1. The chambers might be located out of doors to avoid one thickness of glass, shading by roof rafters and supports, radiated heat from cement floors, soil benches, and the like. 2. The use of clear plastic materials as a substitute for glass would permit lighter construction, easier access to plants, and the use of the entire sun's spectrum for illumination. 3. Built-in heating, humidifying, and plant watering devices. 4. Employment of a heat exchanger in each chamber of the type described would improve refrigeration efficiency.

[4] Twenty-four seedlings, the largest leaves of which were one-half inch in diameter were spaced two inches apart in each flat.

FIG. 6. Response of 24 Burley (upper) and 24 Turkish (lower) tobacco seedlings after a 2-weeks exposure to temperatures approximating 85° and 65° F. N, "neutral" soil; A, "acid" soil, both infested with tobacco black root-rot fungus (*Thielaviopsis basicola*). The largest leaves of each plant at the beginning of this experiment were one-half inch. Distance between plants was 2 inches.

SUMMARY

Refrigerated plant-culture chambers are described for use in full sunlight between temperatures of 65° and 105° F., and in darkness at temperatures as low as 25° F.

Ordinary commercial refrigeration equipment, apparatus, and materials were employed for simplicity and economy.

The accuracy of temperature control obtainable with the commercial equipment used was within 2° or 3° F. of that desired.

The principle of design, adopted for these chambers, may be described as the movement of low-velocity air at a stated temperature across surfaces exposed to light radiation.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE, MISS., AND
VIRGINIA AGRICULTURAL EXPERIMENT STATION,
TOBACCO RESEARCH LABORATORY,
CHATHAM, VIRGINIA.

CONTROL OF SEEDLING DISEASES OF SUGAR BEETS IN MONTANA[1]

M. M. Afanasiev and H. E. Morris[2]

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Seedling diseases have considerable importance in the growing of sugar beets, especially on the heavy irrigated soils of Montana. Young beet seedlings are usually attacked by different pathogenic organisms either at the time when they emerge from the seed balls or later when they have emerged above ground.

Three more or less distinct types of seedling diseases of sugar beets have been described by Coons and Stewart (3). Seedlings with the first type of disease show a browning and blackening of the hypocotyl and root. The discoloration usually shows above the ground and the killing of the seedlings varies from fairly rapid to very slow. A plant may frequently show its hypocotyl completely blackened. The cotyledons, however, remain turgid and green and, on examination, the vascular region of the plant appears to be the only part not affected. *Phoma betae* is chiefly associated with this type of disease and is usually seed-borne.

Seedlings affected by the second type of disease wilt promptly, but usually show no marked discoloration. Such plants are characterized by a brown decayed region in the root, and the central vascular region is discolored far in advance of the external lesions. The lesions have a water-soaked appearance in contrast to the dry, black areas typical of the first type of disease. Twenty-four hours after the first indication of wilting appears the seedling is almost completely decayed. *Pythium* spp. and also other soil-borne phycomycetous fungi are associated with this type of disease.

In the third type of beet seedling disease, the leaves usually show a green or blue-green and the stem a lemon-yellow color. The seedlings grow slowly and, in general, show evidence of malnutrition. The taproot usually is found decayed at the tip, and rootlets appear, apparently, to replace the primary root. *Rhizoctonia* spp., which are also soil-borne, are chiefly responsible for this type of disease.

Most of the beet seedling diseases occurring in Montana resemble more closely the first type, while the second and third types occur only to a limited extent. Up to the present, however, it has not been proved that only *Phoma* is responsible for seedling diseases in this State.

Seedling diseases of sugar beets, at least from the standpoint of the causal organisms, usually are of a complex nature and often several organisms may be responsible for the disease. A slight difference in the composi-

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tion of the soil, its temperature and other factors may be responsible for a variation in the pathogens responsible for diseases of crops (7).

The seedling-disease problem is complicated by the fact that it is rather difficult to isolate from seedlings of the sugar beet the organism mainly responsible for the diseases. Usually there are present, with the primary organisms, secondary ones that grow much faster on ordinary media used for isolations than do the pathogens responsible for the disease. Coons and Stewart (3) found the same difficulties in obtaining isolations of the pathogens responsible for seedling diseases. Numerous isolations from diseased seedlings in Montana were made and the results of the detailed studies on them will be reported in a subsequent paper.

Previous investigations of seedling diseases of sugar beets conducted in the rotation plots at the Huntley Branch Station, in Montana, indicated that the occurrence of these diseases is closely associated with the productive power and physical conditions of the soil, and with the other crops grown in the rotations. Weather and many other factors also may have their effect on the diseases.

SOIL AND SEED SANITATION

Soil and seed-treatment experiments were conducted in 1939 and 1940 at the Huntley station to secure additional data on the relationship between occurrence of seedling diseases of sugar beets and their environment and, also, to develop measures for the control of these diseases.

The land selected for the experiments had been alternately cropped for a number of years with barley and sugar beets, and was so depleted of its fertility that, in 1938, only 6.76 tons of beets per acre were produced.

Seed Treatments

To study the effect of disinfectants on the control of seedling diseases, one 20-lb. lot of sugar beet seed was treated with 4 oz. of Ceresan and another 20-lb. lot with 1 oz. of New Improved Ceresan by thoroughly mixing the disinfectants and seeds in a closed container.

Soil Treatments

The soil amendments were nitrates (N), phosphates (P), manure (M), and lime ($Ca(OH)_2$), applied in the following combinations:

1. NPM; 2. $\frac{N}{2}$PM. In this treatment half of the nitrates were applied at the time of planting and the other half as a side dressing immediately after thinning. 3. NP; 4. $\frac{N}{2}$P. Nitrates were applied in the same way as in 2. 5. $\frac{N}{3}$P. In this treatment, one-third of the nitrates were applied at the time of planting, one-third as a side dressing immediately after thinning, and the remaining one-third about a month later. 6. MP; 7. N; 8.

P; 9. M; 10. $Ca(OH)_2$; 11. NPM + $Ca(OH)_2$; 12. NP + $Ca(OH)_2$; 13. N + $Ca(OH)_2$; 14. P + $Ca(OH)_2$; 15. M + $Ca(OH)_2$; 16. Check.

In both years the nitrogen used was in the form of $Ca(NO_3)_2$, which was applied at the rate of 250 lb. per acre, and contained 38.75 lb. of N, while the phosphorus was a treble superphosphate for which the application rate was 175 lb. per acre, containing 80.50[3] pounds of P_2O_5. Lime in the form of $Ca(OH)_2$ was used and applied at the rate of 1000 lb. per acre. In 1939 manure was applied on the basis of 14.75 tons per acre, and, in 1940, on the basis of 22 tons per acre.

Soil treatments were used in 3 randomized replications, so that, altogether, there were 48 individual plots, each consisting of 3 randomized rows

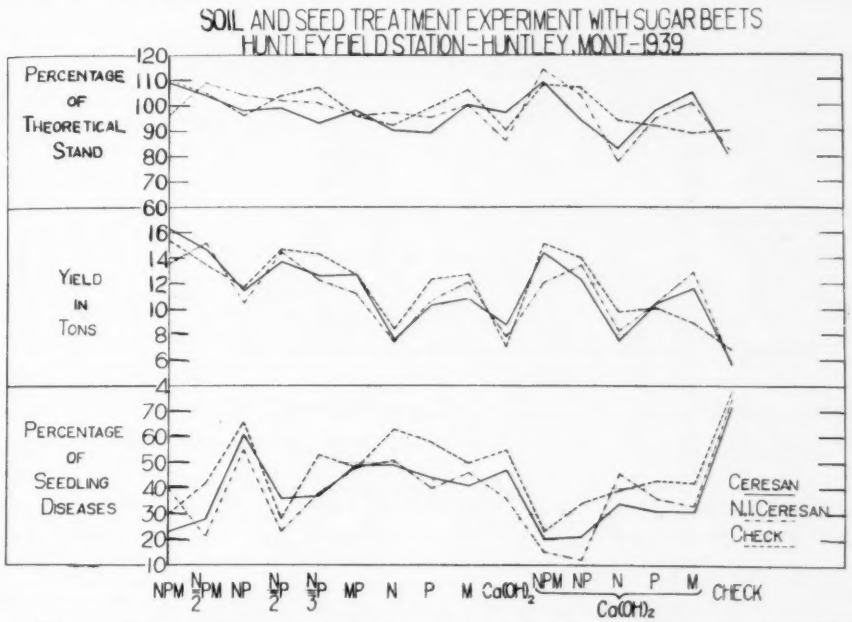

FIG. 1. The percentage of theoretical stand, ton yield, and percentage of seedling diseases of sugar beets obtained from soil and seed-treatment experiments conducted in 1939 at Huntley (Montana) Field Station.

of beets, 125 feet long. One row was planted with seeds treated with Ceresan, another with seeds treated with New Improved Ceresan, and the third with untreated seed. One buffer row was planted between each 3-row plot.

Preparation of Land

The land was plowed in the fall of 1938, double-disked and harrowed in the spring of 1939, after which lime and manure were broadcast and the soil was cultivated. The rows where sugar beets were to be planted were marked, and treble superphosphate and calcium nitrate were applied by hand in strips 3 to 4 inches wide in the center of these rows; these strips were lightly cultivated and the rows seeded. The same procedure was fol-

[3] According to the guarantee by the manufacturer.

lowed for the 1940 crop as in 1939, except that manure was applied in the fall of 1939 just before plowing. A Planet Junior hand planter was used for seeding in 1939 and a regular beet planter in 1940. The early part of the spring of 1939 was so dry that it was necessary to irrigate all beets before emergence. Later, however, there was abundant rain (7.5 inches) between May 20 and June 20. In 1940 there was sufficient spring rainfall to make early irrigation unnecessary.

Disease Readings

Sugar-beet seedlings in large numbers were examined for disease in 1939 and 1940 when the plants were in about the six-leaf stage, when beet-seedling diseases usually are at their maximum intensity. Preemergence diseases

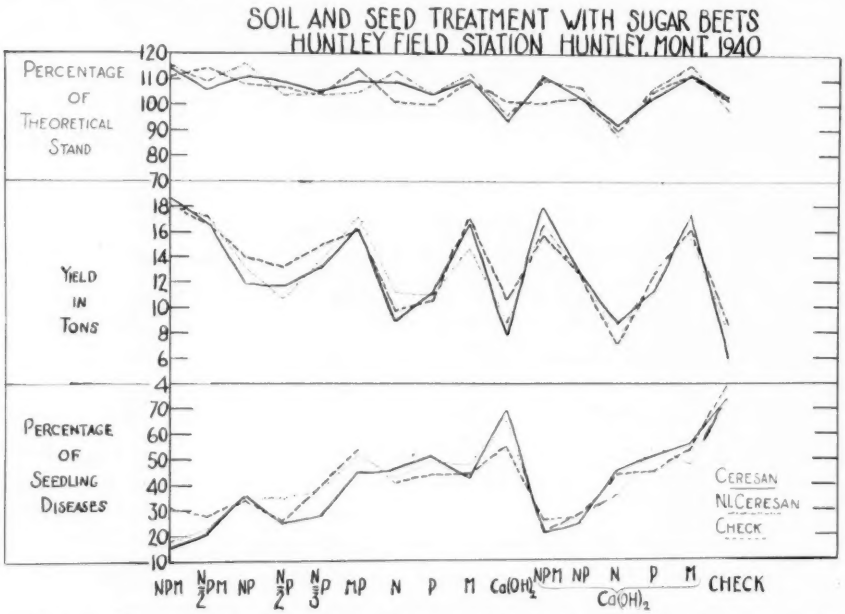

FIG. 2. The percentage of theoretical stand, ton yield, and percentage of seedling diseases of sugar beets obtained from soil and seed-treatment experiments conducted in 1940 at Huntley (Montana) Field Station.

usually were responsible for only a slight loss. At harvest each row was dug, its tops and roots were weighed separately, and disease readings recorded. The results of the disease readings and the yield and stand for each individual soil and seed treatment for 1939 and 1940, respectively, are shown in figures 1 and 2 and tables 2 and 3. The data represent the averages of 3 replications.

EXPERIMENTAL RESULTS

Seed Treatments

The seed treatments (Table 1) had but slight effect on the seedling diseases, on the yield or on the stand of the sugar beets as compared to the check.

TABLE 1.—*The average results from the different seed treatments on all kinds of soil treatments in 1939 and 1940*

Seed treatments	Seedling diseases		Yield		Stand	
	1939	1940	1939	1940	1939	1940
	Per cent	*Per cent*	*Tons*	*Tons*	*Per cent*	*Per cent*
Ceresan	38.9	40.9	11.30	12.92	96.6	105.9
N. I. Ceresan	38.4	41.6	11.10	13.03	97.5	106.4
Check	46.8	41.8	11.70	13.38	99.1	105.2

The Effect of Soil Amendments, Singly and in Combinations, on the Amount of Seedling Diseases, Yield, and Stand of Sugar Beets

Sugar beets planted in plots treated either with nitrates or phosphates, separately or in combinations with $Ca(OH)_2$, had a considerable amount (av. 40.5 to 48.6 per cent) of seedling diseases, fair yields (av. 8.2 to 11.3 tons) the lowest being in the plots treated with nitrates alone (av. 8.9 tons) and in combination with $Ca(OH)_2$ (av. 8.2 tons), and, in general, they produced relatively poor stands.

Four different plots were treated with equal amounts of nitrates and phosphates, the only difference being in the method of applying the former, while one of the plots also had an application of $Ca(OH)_2$. The amount of seedling disease in these 4 plots in 1939 and 1940 was highest (60.7 and 34.9 per cent) in the plots where all nitrates were applied at seeding time while the lowest (22.2 and 26.8 per cent) followed use of nitrates in combination with $Ca(OH)_2$. In both years, less disease was recorded in plots in which nitrates were divided into 2 applications (29.2 and 28.6 per cent) than in those with 3 applications. In the latter the percentage of seedling disease (42.8 and 34.9 per cent) closely approached that of the plots in which all the nitrogen was applied at seeding time. All of these plots showed less disease than did those to which nitrates and phosphates were applied separately. There was little variation in yield and stand in these plots in both years, but they were slightly higher than those where either nitrogen or phosphates were used alone.

Sugar beets grown in the plots treated with manure, manure and $Ca(OH)_2$, and manure in combination with phosphates showed a considerable amount (average 45.5 to 49.1 per cent) of seedling diseases in both years. The yields and stands in 1940 were considerably higher than those in 1939.

Sugar beets in plots treated with either a combination of nitrates, phosphates and manure, or this combination with $Ca(OH)_2$; or with nitrates, phosphates and manure, where the nitrates were divided into two applications, manifested the minimum of seedling disease (av. 21.0 to 27.1 per cent), the highest yields (av. 15.3 to 16.7 tons per acre) and a very good stand of beets of all treatments in both years. The yields of nearly all these plots in 1940 were considerably higher and the amount of disease considerably

TABLE 2.—*Experiments with sugar beets showing the amounts of seedling diseases, theoretical stand, and yield per acre*[a] *1939*

Soil treated with	Percentage seedling diseases from seeds treated with				Yield in tons per acre from seeds treated with				Percentage theoretical stand[b] from seeds treated with			
	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average
NPM	22.8	39.6	29.9	30.7	16.27	13.57	15.39	15.1	109.3	95.7	110.1	105.0
$\frac{N}{2}$PM	27.6	20.7	41.9	30.1	14.74	15.23	13.49	14.5	104.3	108.5	105.1	106.0
NP	61.4	54.9	65.9	60.7	11.45	10.53	11.69	11.2	97.9	103.5	96.3	99.2
$\frac{N}{2}$P	36.3	23.0	28.3	29.2	13.66	14.47	14.67	14.3	98.9	102.4	104.3	101.9
$\frac{N}{2}$P	37.4	38.2	52.8	42.8	12.58	12.25	14.27	13.0	93.1	101.3	106.9	100.4
MP	48.9	47.9	47.5	48.1	12.65	11.20	12.67	12.2	98.1	95.8	95.7	96.5
N	48.5	51.2	62.5	54.1	7.56	7.43	8.35	7.8	89.9	96.8	92.3	93.0
P	43.7	39.5	57.6	46.9	10.30	10.70	12.32	11.1	88.8	95.2	98.7	94.2
M	41.1	46.2	50.3	45.9	10.77	12.10	12.69	11.9	100.0	100.3	105.8	102.0
$Ca(OH)_2$	47.3	36.1	54.9	46.1	8.69	7.81	7.04	7.8	96.5	85.6	89.9	90.6
$NPM + Ca(OH)_2$	19.5	15.1	23.1	19.2	14.43	12.13	15.07	13.9	109.1	114.1	108.3	110.5
$NP + Ca(OH)_2$	20.9	11.9	33.8	22.2	12.25	13.43	13.99	13.2	93.9	104.3	106.9	101.7
$N + Ca(OH)_2$	33.5	46.3	38.9	39.6	7.48	8.31	9.78	8.5	83.3	78.1	94.1	85.2
$P + Ca(OH)_2$	30.7	36.4	42.7	36.6	10.37	10.54	10.09	10.3	98.1	94.7	92.0	94.9
$M + Ca(OH)_2$	30.9	32.9	41.5	35.1	11.62	12.86	8.91	11.1	105.1	101.3	88.8	98.4
Check	72.4	75.1	77.7	75.1	5.72	5.64	6.80	6.1	78.9	82.4	89.6	83.6

[a] All figures represent averages of three replications.
[b] Based on stand of 26,136 per acre.

TABLE 3.—*Experiments with sugar beets showing the amounts of seedling diseases, theoretical stand, and yield per acre*[a]
1940

Soil treated with	Percentage seedling diseases from seeds treated with				Yield in tons per acre from seeds treated with				Percentage theoretical stand[b] from seeds treated with			
	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average	Ceresan	New Impr. Ceresan	Check	Average
NPM	16.2	18.1	31.0	21.8	18.57	18.00	18.12	18.23	114.1	115.2	110.9	113.4
$\frac{N}{2}$ PM	21.2	22.6	28.2	24.0	16.67	17.18	16.65	16.83	105.9	108.5	113.9	109.4
NP	36.1	34.9	33.6	34.9	11.91	13.14	13.98	13.01	111.2	116.0	107.7	111.6
$\frac{N}{2}$ P	25.4	34.5	26.0	28.6	11.68	10.72	13.16	11.85	108.8	103.5	106.9	106.4
$\frac{N}{3}$ P	27.8	37.8	39.2	34.9	13.23	13.67	14.93	13.94	104.8	103.5	104.3	104.2
MP	44.5	51.7	53.7	50.0	16.33	17.16	16.08	16.52	109.3	104.5	113.9	109.2
N	45.7	43.1	40.6	43.1	8.86	11.19	9.70	9.92	108.5	113.3	101.3	107.7
P	51.3	49.2	43.8	48.1	11.22	11.02	10.51	10.92	104.3	103.7	99.7	102.6
M	43.1	48.1	43.9	45.0	16.69	14.65	17.00	16.11	110.1	112.0	108.5	110.2
$Ca(OH)_2$	69.4	65.1	55.2	63.2	7.78	8.74	10.61	9.04	93.9	96.3	101.3	97.2
$NPM + Ca(OH)_2$	21.4	21.1	25.6	22.7	17.89	16.61	15.78	16.76	110.7	110.1	101.3	107.4
$NP + Ca(OH)_2$	25.0	28.3	27.0	26.8	12.70	12.64	12.70	12.68	102.9	107.2	103.2	104.4
$N + Ca(OH)_2$	45.2	35.2	43.6	41.3	8.58	7.42	7.11	7.70	92.0	88.3	89.6	90.0
$P + Ca(OH)_2$	51.3	54.9	44.6	50.3	11.44	12.99	12.74	12.39	103.2	106.7	106.1	105.3
$M + Ca(OH)_2$	56.4	47.6	53.6	52.5	17.40	15.53	16.22	16.38	112.3	115.7	111.7	113.2
Check	74.1	73.9	78.9	75.6	5.78	7.81	8.81	7.47	101.6	97.6	102.4	100.5

[a] All figures represent averages of three replications.
[b] Based on stand of 26, 136 per acre.

lower than in 1939. The stand of beets was just about the same in both years.

Sugar beets, grown in the plots treated with $Ca(OH)_2$, showed, in both years, a very high amount of seedling disease, low yields, and relatively low stands. When identical plots, treated with $Ca(OH)_2$ are compared with those receiving no treatment, the 1939 results show that sugar beets planted in the plots treated with lime had considerably less seedling disease than those in non-treated plots, but in 1940 there was very little difference in the percentage of such disease in all plots. The yield and stand of beets, however, were about the same in both years for the plots treated and non-treated with lime.

Lime was added with some of the soil amendments, because the heavy clay-loam soil of the Huntley area is essentially free of carbonates. The lime was applied to improve the physical conditions of the soil, thus making it more porous, giving better aeration, and facilitating greater microbiological activity, including nitrificaton. Lime added to the soil also may serve as a readily available source of calcium.

The check plots showed the highest percentage of seedling disease (av. 75.4 per cent), lowest yields (av. 6.9 tons), and stands of all plots used in both years. The percentage of seedling disease in the check was several times higher than in the plots with the best soil treatments.

Those plots receiving the most nearly complete soil amendment each year showed a tendency toward a decrease in percentage of seedling disease and an increase in yield in 1940, as compared with 1939. The check plots, the plots treated with $Ca(OH)_2$ only, and those given a single soil amendment showed, in general, an increase in seedling disease. Contrary to expectation, there was a slightly greater increase in yield and in stand in 1940 than in 1939. There undoubtedly are present two parallel and hardly separable effects of fertilizers on sugar beets, one on the control of the seedling disease of beets and the other on the yield.

In the type of root rots occurring in Montana, although many of the diseased seedlings recover, there usually is a high mortality. Damage to the crop, therefore, results not only from reduced stand, but to some extent from retarded growth of plants of low vigor. In spite of a comparatively good stand of beets in the plots showing an abundance of seedling disease, the yields of these plots were poor because of small beets.

DISCUSSION

An analysis of the results of these experiments indicates that certain conditions characterizing these heavy Montana soils are responsible for the occurrence of these root rots of sugar-beet seedlings. It is evident from the 2 years' results that the plots receiving the most complete and balanced soil amendments produced the minimum of diseased seedlings, the largest yields, and, comparatively, the best stands. The fact that the seedling root rots can be controlled by an application of a complete and balanced fertilization

shows that the disease factor itself is not the most important, but that the soil conditions are of first significance. The only exception seems to be in the case of the plots treated with manure, and with manure and phosphates, which, in spite of a considerable showing of seedling disease, yielded well, especially in 1940. This apparently contradictory situation may possibly be explained on the basis that sugar beets require a generous amount of easily available nitrogen in their early stage of development. Manure, with potentially a large supply of nitrogen, may have only a small part of it in nitrate form at the time of the early development of the beets. Soil temperatures are not then favorable for nitrification; so there may be a deficiency in nitrates, even in the soils sufficiently fertilized with manure. Later in the season, with increase in nitrification, the beets, even if affected with root rots, will have a better chance of survival and a more normal development than beets grown in soils of low fertility.

It is well known that sugar beets depend for their growth on a large amount of easily available nutrients; that the feeding area of beet roots in their early stages of development, both horizontally and vertically, is rather limited (10), that beet seedlings, up to the 6-leaf stage are very susceptible to seedling diseases, and that they later become more resistant (4, 5). This resistance, in a broadly interpreted sense of the term, is possibly attributable to the fact that as soon as the sugar beets increase their root systems and, consequently, their feeding areas, more nutrients are absorbed. In well-fertilized soils there are adequate nutrients for the young beets, consequently, they are resistant to root rots at a very early stage as compared with beets grown in soils of low fertility. Indications are that development of black root of sugar beets is facilitated by soils of low fertility with a small amount of easily available nutrients. Weather and many other factors also may influence these diseases, but it seems that soil nutritional factors have the greatest effect on the occurrence of certain soil-borne diseases of plants, a fact emphasized by several investigators (1, 2, 8, 9).

Furnishing readily available plant food to the young sugar beet while it is becoming established and its root system is small, appears to be a significant factor in making the plant more resistant to the invasion by some soil or seed-borne pathogens. Proper and complete soil fertilization also may affect the root-rot organism or organisms of young sugar beets. Sanford, in his studies of *Rhizoctonia solani* (6), reports that there is evidence indicating that conditions favorable to marked vegetative growth of the pathogen tend to depress its virulence.

It is quite possible that the pathogenicity of the root-rot fungus or fungi is noticeably less in soils well supplied with nutrients than in those poorly supplied. It is also possible that soil containing a considerable amount of nutrients stimulates the development of saprophytic soil organisms, which, through competition, may reduce the number of parasitic organisms in the soil.

SUMMARY

Seed treatments alone were found to be only slightly beneficial in controlling seedling diseases of sugar beets.

Soil treatments, regardless of seed treatments, proved highly important to the control of seedling diseases of sugar beets.

Plots with the most complete soil treatments, namely NPM, $\frac{N}{2}$PM, $\frac{N}{2}$P, NPM + $Ca(OH)_2$ and NP + $Ca(OH)_2$ produced the minimum of seedling disease, and the highest yields and stands.

Plots treated with NP, $\frac{N}{3}$ P, and MP had fairly good yields and stands, yet had considerable amounts of seedling diseases.

The plots with unbalanced soil amendments (N, P and M), all showed a high amount of seedling disease; the yields and stands also were poor, except for plots treated with manure.

Check plots and those treated only with $Ca(OH)_2$ had the highest amounts of seedling diseases and poorest yields and stands.

Seedling diseases of sugar beets in heavy, irrigated Montana soils can be efficiently controlled and at the same time good stands and high yields of sugar beets obtained by creating conditions that will promote a rapid and healthful development of young sugar beets through sufficient and balanced fertilization and improvement of the physical condition of the soil.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
MONTANA AGRICULTURAL EXPERIMENT STATION,
MONTANA STATE COLLEGE,
BOZEMAN, MONTANA.

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NOTES ON CLITOCYBE ROOT ROT OF BANANAS AND OTHER PLANTS IN FLORIDA

ARTHUR S. RHOADS[1]

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This paper presents an account of the occurrence of Clitocybe root rot of bananas in Florida, an abstract of which was published in 1932 (6), and also records the extreme destructiveness of this disease on other exotic plants, chiefly subtropical, on the same property on which it proved so destructive to bananas.

On January 24, 1931, the writer visited a property at Artesia, Brevard County, Florida, to investigate a suspected case of Clitocybe root rot of banana plants. An inspector of the State Plant Board had previously collected and sent for diagnosis the base of one plant to the Florida Agricultural Experiment Station at Gainesville. This property is located between Cocoa Beach and Canaveral on the barrier reef along the East Coast. The soil is Palm Beach sand, and, prior to clearing, was covered with a dense hammock forest in which live oak trees were predominant. Although the land had been cleared for several years, many oak roots had been left in it and the situation was a very favorable one for the development of the Clitocybe root-rot fungus.

Several banana plants of 3 varieties were found attacked by the disease in question. An examination of the interior tissues of the bases of the attacked plants revealed an unusually luxuriant development of the characteristic whitish mats of mycelium and rhizomorphs of the fungus permeating not only the fibrous tissues of the corm but also the base of the pseudostem (Figs. 1 and 2). The remains of an old cluster of the toadstools of *Clitocybe tabescens* (Scop.) Bres. were found at the base of one of the attacked plants.

Peeling off the outer dead leaf bases of attacked plants immediately disclosed the extent of the invasion of the bases of the pseudostems by the fungus. All the attacked plants examined were characterized by a watery, dark-brown zone involving the basal portion invaded by the whitish rhizomorphs and extending upward for some distance beyond the general level of the invaded portion in a series of irregular streaks (Fig. 2). This basal discoloration of the pseudostem ranged from a few inches to about a foot in height.

In dissecting attacked plants by carefully peeling off the fleshy leaf bases many varying and intricate patterns of rhizomorph formation were apparent. These not only penetrated the spongy, septate tissues of the succulent leaf bases but frequently grew out through the surface and developed between the compact concentric arrangement of leaf bases composing the pseudostem. They ranged from numerous, more or less branching thread-

[1] Formerly Plant Pathologist of the Florida Agricultural Experiment Station, in charge of the Citrus Field Laboratory at Cocoa.

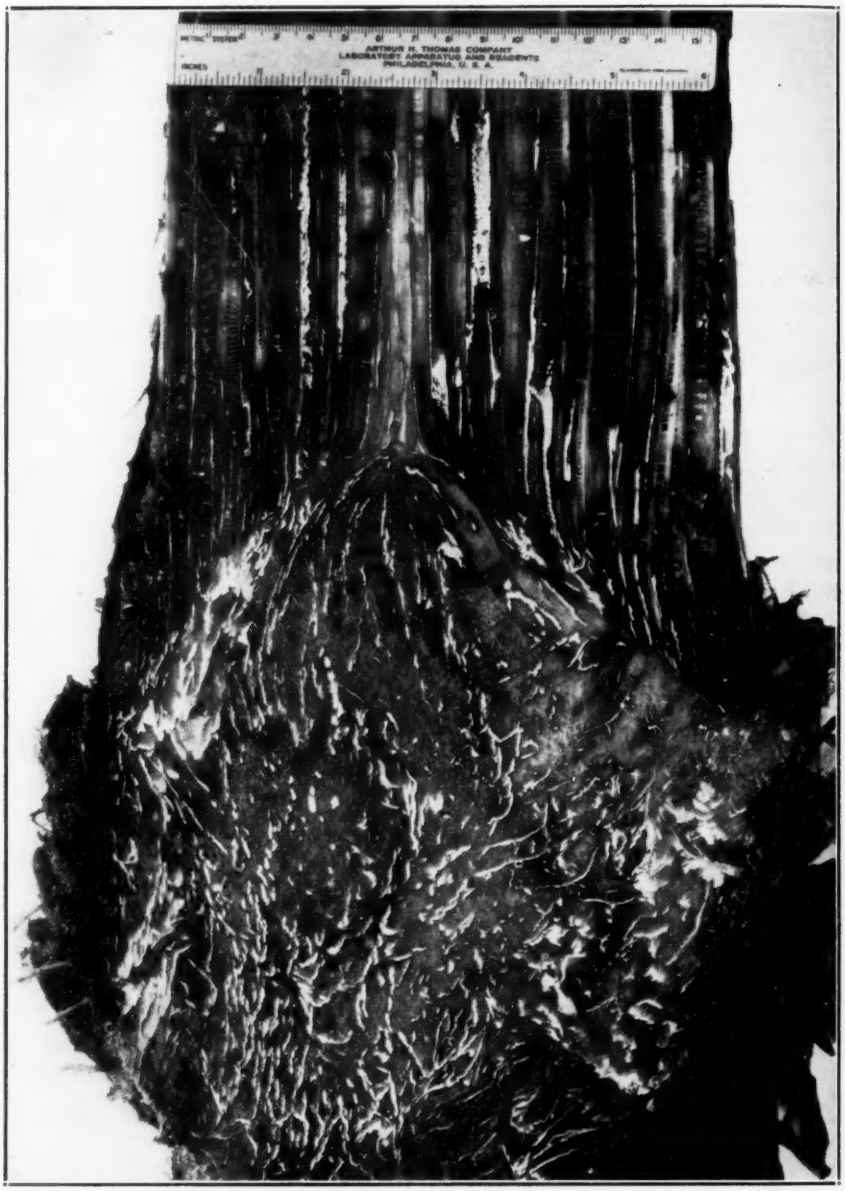

FIG. 1. Longitudinal section through corm and base of pseudostem of banana plant recently killed by Clitocybe root rot, showing whitish mycelial mats and rhizomorphs irregularly distributed throughout the tissues.

FIG. 2. Base of pseudostem of banana plant recently killed by Clitocybe root rot, with outer leaf bases peeled off, showing the watery, dark-brown zone characterizing the portion invaded by whitish rhizomorphs of the fungus and extending upward in a series of irregular streaks.

like rhizomorphs to sometimes greatly broadened, irregular, thallus-like, whitish rhizomorphic sheets with the margins dividing into a series of simple or branching, thread-like rhizomorphs (Fig. 3, A). Sometimes the broad thallus-like rhizomorphs were irregularly lobed and, particularly where of recent growth, had developed little or no thread-like branching from the margins (Fig. 3, B). The broad, thallus-like type of rhizomorphs sometimes exhibited a peculiar perforate character, which is apparent in figure 3. The reason for this character is unknown, but it has been observed frequently in mycelial sheets of *C. tabescens* developed between the bark and wood of a wide variety of hosts, including both hardwood and coniferous trees, and appears to be a characteristic feature of this fungus. The rhizomorphic strands and sheets thus developed in and between the banana leaf bases were essentially counterparts of the same structures commonly produced in luxuriantly growing flask cultures of the fungus. However, according to extensive observations over a period of several years in various parts of Florida, the black, rounded or flattened, cortical and hypogeal rhizomorphs, so commonly produced by the closely related *Armillaria mellea*, are not produced by *C. tabescens*.

Pure cultures of *C. tabescens* were isolated subsequently from the interior tissues of each of two different plants. Subcultures of these were carried to fruition in comparison with isolations of this fungus from various other plants attacked by Clitocybe root rot, including some from the same property.

Upon transmitting to Gainesville specimens and the results of his findings, the writer was informed that a specimen of banana plant with the same trouble had been received from Nocatee, DeSoto County, Florida, on December 12, 1929, though it had not been so identified at the time. A portion of the latter specimen had been preserved, so that it was available for comparison. Unfortunately, however, no record was made of the name of the person who sent this specimen; otherwise a special effort would have been made to visit this property. On May 18, 1934, Mr. Erdman West, Mycologist of the Florida Agricultural Experiment Station, collected at Gainesville a specimen of Orinoco or "horse" banana, which he found attacked by Clitocybe root rot. This occurred in the yard of an apartment house where loquats and poinsettias in the same vicinity had died previously, presumably from the same trouble.

On September 1, 1931, Clitocybe root rot was found to be spreading on the banana plants at Artesia. A number of attacked plants were dug up for examination and all exhibited the same characteristic symptoms as those examined in January. A miniature cluster of young sporophores was found developing under the outer leaf base at the base of one plant. This was an exact counterpart of those that had developed on many occasions in cultures of the fungus from various sources. The stump of this plant was transported to Cocoa to see if further growth of the sporophores could be induced, but they shriveled after a few days.

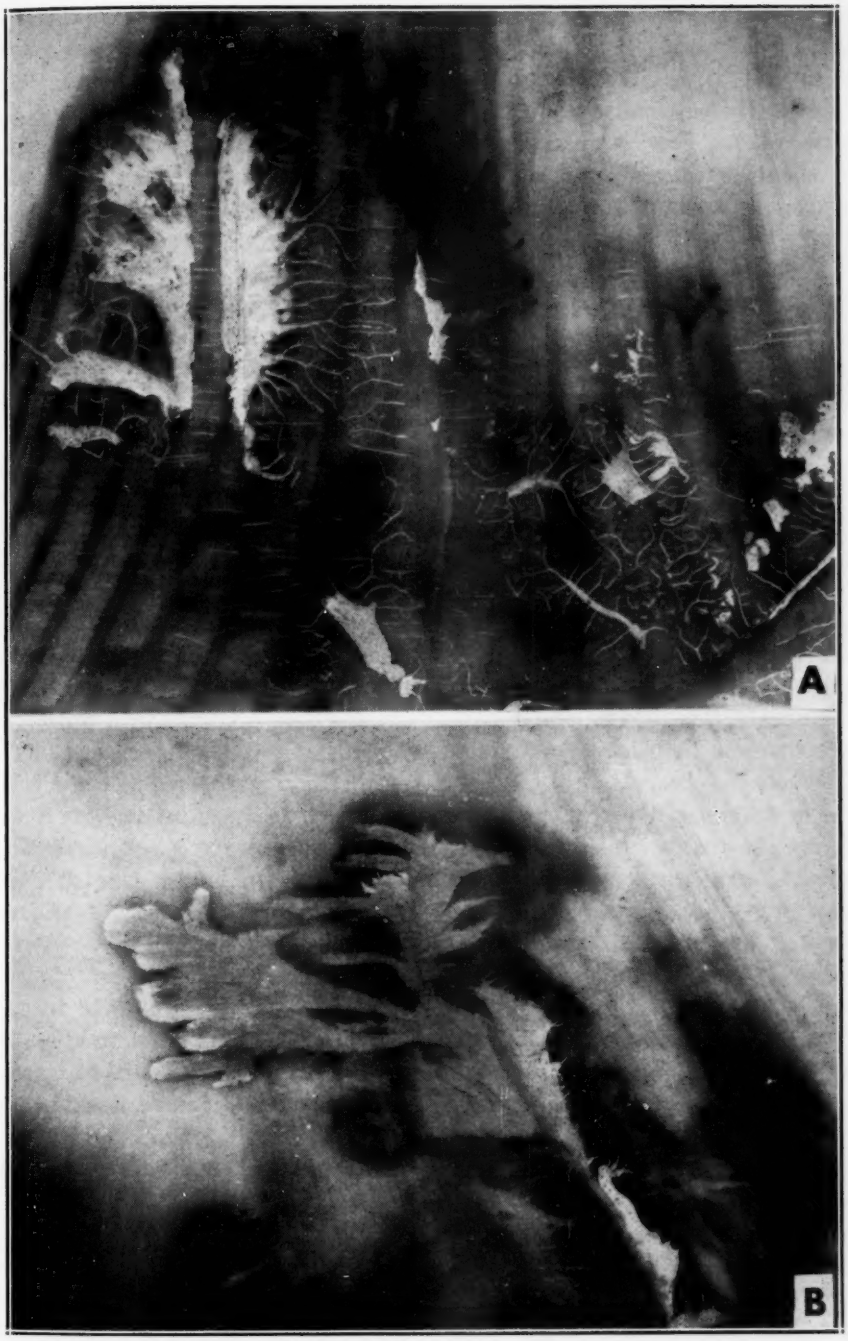

FIG. 3. Inner surfaces of banana leaf bases, flattened, showing various types of imbedded and superficial rhizomorphs. A. Rhizomorphs ranging from thread-like to thallus-like formations with a perforate character, the strip torn through broad one at left adhering to outer surface of next leaf base. B. Irregular branching, thallus-like rhizomorph with perforate character developed within the septate tissues, showing dark-brown, watery zone developing in advance of invasion. Natural size.

In checking over the banana mortality with the grower, it was estimated that he had already lost one stool of the Cavendish or Chinese (*Musa cavendishii* Lamb), 4 of the Hart's Choice or Lady Finger (*M. sapientum*, var. *champa* Baker), and 4 or 5 of the Orinoco or "horse" banana (*M. paradisiaca* L.). These were all planted close together. The grower stated that he also had lost a stool of the Red Jamaica variety, planted some distance away; but, since this had been removed and destroyed, it was impossible to verify this instance as an additional case of root rot. An investigation of the mode of spread of the disease showed that when plants became infected the fungus gradually spread through the corms and up first one shoot and then another, even attacking shoots but 2 to 3 feet high, and eventually killing the entire stool. Cultures of *C. tabescens* were isolated from the interior tissues of a dead young sucker of Hart's Choice banana and carried to fruition.

Unfortunately, banana plants on which inoculation experiments could be performed with pure cultures of *Clitocybe tabescens* associated with the corm rot here reported were not available. However, extensive experience with the destructive nature of this widely distributed parasite, both on the property at Artesia and those at numerous other points in Florida, leave no doubt about this fungus being the cause of the disease reported. In fact, the writer (7, 8) has produced infection and death of Australian pine (*Casuarina equisetifolia*) trees in Florida through inoculations with pure cultures of this fungus, and Plakidas (5) has done likewise with pineapple pear trees in Louisiana.

Since the observations here reported were made, the occurrence of a corm rot of bananas, due to *Clitocybe* sp., has been reported from New South Wales, Australia. In 1934, Noble (3) mentioned dry rot (*Clitocybe* sp.) as one of the diseases affecting Cavendish bananas in newly cleared lands, though in a previous report (2) he stated that "Banana root rot (*Armillaria mellea*) was reported in some areas." In a list of plant diseases recorded in New South Wales, prepared by him and others (4, p. 27), both diseases are listed as occurring on banana. *Armillaria mellea* is followed by the notation "Root Rot. 1917. N. Coast," while *Clitocybe* sp. is followed by the notation "Corm Rot. 1929. N. Coast." Whether both root rots of banana occur or the disease was first attributed to *Armillaria mellea* and later to *Clitocybe* sp. is not clear.

A brief account of corm rot of bananas was given in the following year by Magee and Eastwood (1), who state that it has been known for some years but has become of greater importance with the extension of banana planting to hardwood forest lands. The disease is said to be encountered but rarely in scrub lands (brushwoods), but, in forest and bastard scrub country, up to 300 to 400 stools have been lost at times in a 10-acre plantation. Corm rot is considered to be primarily a disease of new plantations on recently cleared land. The Australian disease results in a "dry brown corm rot, with white fungous threads interwoven throughout." The symp-

toms of the plant decline are described and appear to agree closely with those of the banana root rot here reported from Florida. A species of *Clitocybe* is said to be one of the most commonly associated parasites, but, unfortunately, the species was not determined—a point of vital interest. So far as the writer knows, *C. tabescens* has never been reported as occurring in Australia.

The owner of the property at Artesia, who had an ornamental nursery and a collection of miscellaneous trees, including several rare subtropical ones, proved to have had considerable experience with plants. Upon being informed as to the cause and nature of the disease attacking his bananas on the occasion of the writer's first visit in January, 1931, he mentioned that he had lost an apple and an apricot tree some time before, due, he thought, to borers. The apple tree was 5 years old from the seed and the apricot tree was a budded one purchased from a nurseryman 4 years previously. They were near each other and about 200 feet from the attacked banana plants. Upon examination, both showed the characteristic mycelial sheets of the Clitocybe root rot fungus between the bark and the wood at the base and were dug up for cultures. *C. tabescens* was isolated subsequently from the apple tree and carried to fruition, but was not secured from the apricot tree, as it was pretty well desiccated. The writer was then informed that a guava (*Psidium guajava*) and a Java-plum (*Eugenia jambolana*) also had died. The latter had been cut off, but the stump remained. Upon examination, it was found that both trees were attacked by Clitocybe root rot.

On the occasion of the writer's second visit in September, 1931, the disease had spread to the one remaining trunk of the guava clump that was found partly dead in January. This appeared quite healthy and had a fair crop of fruit nearing maturity. However, examination of the base revealed the characteristic development of the Clitocybe root-rot fungus under the bark, encircling the trunk up to a point about 18 inches high, where the trunk forked, and continuing up one division to a point 2 feet high. The invaded bark at the upper limit of the lesion was characterized by a slight sunken condition, which demarked the progress of the disease. While the life of this trunk did not appear to be affected, the top wilted and died shortly thereafter.

A large specimen of the trumpet-tree (*Cecropia palmata*) also was found attacked by Clitocybe root rot. The top was in good condition, but the fungus had invaded the bark of the trunk and the roots at one side of the base. The grower had recently cut away the dead bark at the base and had removed a large dead lateral root that remained lying by the tree. This root had an abundant development of mycelium under the bark, resulting in a soft white decay of the delignifying type. Another smaller specimen of trumpet-tree, planted in another portion of the property, also was found to have been killed by root rot.

A magnificent example of rose-apple (*Eugenia jambos*) also was found to be attacked by Clitocybe root rot. A small bark lesion occurred at one

side of the base of the trunk and the removal of the soil at this point disclosed a large dead lateral root. The bark of both contained a luxuriant development of mycelium.

The grower stated that about 2 months previously he had removed a cherimoya (*Annona cherimola*) and a soursop or guanabana (*Annona muricata*), both of which apparently had died from the same trouble. The places where these trees had been dug out were between 7 and 8 feet apart and both were on a 20-foot radius from the infected rose-apple. The grower recalled that about 2 years before he had removed another cherimoya tree, growing about 8 feet from the other. It was learned that the trunks of both the cherimoya and the soursop trees, which had been removed 2 months before, had been dumped into a nearby ditch. The abundant development of the characteristic mycelial sheets of the Clitocybe root-rot fungus, found under the bark of the roots of both these trees, was convincing evidence that they also were victims of this destructive root-rot fungus. At another point on the property a third cherimoya tree, which had died recently from the same trouble, was found. This tree had a bark lesion all around the base and, when dug up, the bark of this lesion and that of the entire root system was found invaded by mycelium. *C. tabescens* was isolated from the roots of this tree and also from a rose bouquet (*Dombeya punctata*) bush that had died from root rot on a neighboring property and carried to fruition in each case. Before leaving this extremely interesting property a young Abundance plum, a large Woodland Margaret rose on its own roots, and a hibiscus (*H. rosa-sinensis*) bush also were found to have succumbed to Clitocybe root rot.

On August 3, 1932, another visit was made to this property and several new plants were found to be attacked by Clitocybe root rot. Among these may be mentioned a Mexican guava (*Psidium molle*) and a Java-plum (*Eugenia jambolana*), both of which had been dead a few months, as well as a large Catalonian jasmine (*Jasminum grandiflorum*) vine that recently had shriveled and died. A 7-inch Carolina laurel cherry (*Laurocerasus caroliniana*) but a foot away from this jasmine next attracted the writer's attention by reason of a profuse basal gumming that extended about two-thirds around the trunk. The dead area of bark, apparent on one side, showed the development of the Clitocybe root-rot fungus. A few feet distant another Carolina laurel cherry of the same size was found with identical symptoms. The root rot had not progressed sufficiently in either case to affect the tops of these trees. In another part of this small property a sapodilla (*Achras sapota*) was found to have shriveled without loss of the foliage at about the time the fruit was maturing. This tree was dug up for examination and nearly all the larger roots were completely invaded by the mycelium of the Clitocybe root-rot fungus.

A physic nut (*Jatropha curcas*) also was found completely girdled by the same fungus, with the bark dead up to an average height of a foot, at which point a pronounced callus formation had developed at the lower mar-

gin of the bark remaining alive. The top of this tree was still living but had comparatively few leaves remaining. The grower reluctantly permitted this tree to be dug up. It was found to have a twin tap root, one division of which had a luxuriant development of the Clitocybe root-rot fungus under the bark. A few concentric and occasional radial cracks occurred in the wood of this root, into which sheets of mycelium had grown. Where the side of the other division of the taproot was in contact with this dead one the bark was found to have become infected—a typical case of the spread of this root disease by contact of a sound root with an infected one. Some of the lateral roots also were found attacked by the root-rot fungus. Pure cultures of *C. tabescens*, with the usual abundant production of whitish rhizomorphs, were obtained from the roots of both this and the sapodilla tree and carried to fruition.

On April 30, 1941, the grower stated that the disease had attacked a litchee (*Litchi chinensis*) about 3 or 4 years previously, but that he had treated it and the tree had since borne good crops of fruit. He further stated that he had found that the recommended procedure of exposing the root crowns of attacked plants to aeration and drying had proved effective in arresting the progress of the disease, and that with this in conjunction with surgical treatment he had been able to save a number of trees attacked by root rot, provided the disease was detected and treatment administered before it had progressed too far.

The high degree of mortality in that portion of this small property devoted chiefly to subtropical plants was attributable largely to the practice of close planting, allowing the diseased trees to remain frequently long after they had died, and incomplete removal of the roots when the trees were finally dug up, thereby increasing the number of centers of infection. As a result, most of the plants enumerated were attacked or killed by the time they had attained an age of from 4 to 7 years. Doubtless a number of other new hosts for Clitocybe root rot might have been determined had the writer continued to have access to this property after 1932. However, circumstances arose to prohibit further inspection of the plantings for the purpose in which he was interested.

SUMMARY

A mushrooom or toadstool root rot of bananas occurring in Florida, caused by *Clitocybe tabescens*, which has been found to be of widespread occurrence in this State and frequently very destructive to a large variety of native trees, fruit trees, ornamental trees, shrubs and vines, is reported.

The symptoms, which are described and illustrated in detail, agree closely with those of a corm rot of bananas reported as becoming increasingly prevalent in New South Wales, Australia, as plantings are extended to newly cleared hardwood forest land, and attributed to an undetermined species of *Clitocybe*.

Notes are also given on the widespread loss of a number of trees and

other plants, mostly exotic and including a number of uncommon subtropical ones, from attack by *Clitocybe tabescens* in the same property where this disease proved so destructive to bananas.

COCOA, FLORIDA.

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THE FUNGICIDAL AND PHYTOCIDAL PROPERTIES OF SOME COPPER XANTHATES

M. C. Goldsworthy, R. H. Carter and E. L. Green

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The copper xanthates are of interest to plant pathologists working with fungicides because they contain both copper and sulphur and are nearly insoluble in water. They are the copper salts of the theoretical xanthic acids $R \cdot OCS_2H$, in which R represents an alkyl organic radical. In water suspensions only small amounts of copper or sulphur appear to be available at any one moment. Our study of their properties was the result of a search for less injurious substitutes for the common orchard fungicides, Bordeaux mixture, sulphur, and lime-sulphur, all of which may cause injury to fruit trees under certain conditions.

The soluble xanthates are made by combining equimolecular quantities of an alkali or alkaline earth metal hydroxide, carbon disulphide, and an alcohol or a compound containing an alkyl hydroxyl grouping. The following example indicates the reactions concerned in the formation of potassium ethyl xanthate:

$$C_2H_5OH + KOH + CS_2 \rightarrow C_2H_5OC(=S)SK + H_2O$$

Cuprous xanthates may be made by the treatment of a solution of an alkali metal xanthate with a solution of a copper salt. Di-xanthogens, also, are produced in the resultant reaction and these were removed from the insoluble precipitates by treatment with acetone.

$$4\ C_2H_5OC(=S)SK + 2\ CuSO_4 \rightarrow 2\ C_2H_5OC(=S)SCu + (C_2H_5OCS_2)_2 + 2\ K_2SO_4$$

For the experiments reported in this paper the cuprous xanthates of the lower alcohols (methyl, ethyl, propyl, butyl, isoamyl),[1] and some of their isomers[2] were made by treating solutions of cupric sulphate with the potassium xanthates of the respective alcohols. The cuprous xanthates precipitate out as finely divided yellowish powders, not wettable in water. Copper ethyl xanthate and copper isoamyl xanthate were prepared for us in larger lots for field trials through the courtesy of the Monsanto Chemical Company, of St. Louis, Mo. The copper content of the samples (Table 1) compared to the theoretical copper content indicated a high degree of purity.

[1] A material sold as isoamyl alcohol was used. The exact content of this compound it not known.

[2] Carter, R. H. Insecticidal and fungicidal compositions. U. S. Patent No. 2150759. U. S. Patent Office, Washington, D. C. 1939.

TABLE 1.—*Copper content of various copper xanthates*

Compound	Percentage copper theoretical	Percentage copper found
Cuprous methyl xanthate	37.2	35.7
" " "		37.4
Cuprous ethyl xanthate	34.4	32.0
" " " commercial[a]		38.6
Cuprous N propyl xanthate	31.6	31.1
" iso " "		33.1
Cuprous N butyl xanthate	29.8	29.7
" " " "		26.2
Cuprous isoamyl xanthate	28.0	27.3
" " " "		29.3
" " " " commercial[a]		28.8

[a] Obtained through courtesy of the Monsanto Chemical Co., St. Louis, Mo.

Table 2 shows the solubility of 5 of these compounds produced in our laboratories and known to be pure. All of the solutions were found to react with chromotropic reagent, a sensitive indicator for dilute concentrations of ionized copper[3] and none gave indications of SH ions when treated with 5 per cent nitroprusside solutions.[4] All, and especially the solutions of the methyl, propyl, and butyl copper xanthates, possessed odors indicating sulphur affinities.

The laboratory tests were conducted according to the methods outlined by Goldsworthy and Green[5] and consist, briefly, in subjecting conidia of *Sclerotinia fructicola* (Wint.) Rehm. (peach brown-rot pathogen) and *Glomerella cingulata* (Ston.) Spaul. and Schrenk (apple bitter-rot pathogen) to 24-hour perfusions with saturated water solutions of the chemicals, and to residues of the chemicals remaining on sprayed glass cover slips after weathering in an apple orchard. Phytocidal tests were conducted on bean, *Phaseolus vulgaris* L. var. Red Kidney, and on apple, *Malus sylvestris* Mill. var. Starking Delicious.

Copper methyl, propyl, and butyl xanthates possess such a penetrating and disagreeable odor that they were not used in field residue tests.

The perfusion and phytocidal tests were conducted in the laboratory and greenhouse with the copper methyl, ethyl, propyl, butyl, and isoamyl xanthates. Table 2 shows the fungicidal value of the saturated solutions of the various copper xanthates against the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*.

All of the saturated solutions were found to be toxic in varying degrees to the conidia of *Sclerotinia fructicola*. Of the 5 tested, copper ethyl

[3] Goldsworthy, M. C., and E. L. Green. Availability of the copper of Bordeaux mixture residues and its absorption by the conidia of *Sclerotinia fructicola*. Jour. Agr. Res. [U.S.] 52: 517–535. 1936.

[4] ———. The fungicidal action of liquid lime-sulfur. Phytopath. 18: 355–360. 1928.

[5] Goldsworthy, M. C., and E. L. Green. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Jour. Agr. Res. [U.S.] 56: 489–506. 1938.

TABLE 2.—*Fungicidal value of saturated aqueous solutions of copper xanthates applied to the condia of S. fructicola and G. cingulata*

Material	Solubility in water in p.p.m.[b]	Hours perfused	Effect on the condia of *S. fructicola*, 7 days old, grown at 75° F.	Sub-growth[a]	Effect on the conidia of *G. cingulata* 5 days old grown at 75° F.	Sub-growth[a]	Growth in controls	
							S. fruticola conidia	*G. cingulata* conidia
				Per cent		*Per cent*	*Per cent*	*Per cent*
Copper methyl xanthate	0.7	6	Few cells plasmolyzed	32	No germination; no apparent injury	82	100	96
		24	All appear plasmolyzed	12	82 per cent germinated	86	100	100
Copper ethyl xanthate	1.78	6	Many appear plasmolyzed	12	No germination	52	100	96
		24	All appear plasmolyzed	0	Mostly all plasmolyzed	11	100	100
Copper propyl xanthate	0.75	6	No apparent injury	86	No apparent injury	92	100	82
		24	Some plasmolysis	16	Some cells appear injured	74	100	100
Copper butyl xanthate	0.62	6	All appear plasmolyzed	12	Many injured	62	100	96
		24	All plasmolyzed	0	" "	48	100	100
Copper iso-amyl xanthate	1.18	6	Some plasmolysis	91	No apparent injury	84	100	82
		24	Many cells injured	12	Some plasmolysis	62	100	100

[a] Perfused conidia transferred after 24 hours to potato-hard-agar and incubated for 24 hours and observed for continued growth.
[b] Parts Cu per million in distilled water.

xanthate and copper butyl xanthate showed the greatest fungicidal value at the end of 6 hours of perfusion; at the end of 24 hours, both prevented germination entirely. Methyl, propyl, and isoamyl xanthates showed the least fungicidal value to the conidia of *S. fructicola*. None of the xanthates was completely effective against the conidia of *Glomerella cingulata*, but all showed some degree of fungicidal value. Copper ethyl xanthate proved to be the most potent of the 5 used against this organism. It is not known whether the fungicidal value of these solutions was due entirely to available copper or to dissolved sulphur. The fungicidal values obtained appear to indicate that they are attributable to the presence of available copper rather than to available sulphur. The varying responses of the conidia of both species are indicative of the presence of copper values. If the results were due to the presence of soluble sulphur it appears very unlikely that the conidia of *G. cingulata* would have responded to the treatments. The data also appear to support the theory that only a portion of the soluble copper was available for the reactions, since none of the actual values approached the theoretical values for the effect of copper.

TABLE 3.—*Fungicidal value of various concentrations of soluble copper, as copper sulphate, applied to the conidia of Sclerotina fructicola and Glomerella cingulata at 74° F.*

Copper concentration	Hours perfused	Effect on the conidia of *S. fructicola*, 7 days old	Subsequent growth	Effect on the conidia of *G. cingulata*, 5 days old	Subsequent growth
			Per cent		*Per cent*
0.25 p.p.m.	6	Some slight injury noted	90	No apparent injury	100
	24	Apparent injury to some	56	" " "	100
0.50 p.p.m.	6	Few have germinated; few appear injured	48	" " "	68
	24	All appear injured	Trace	Many appear injured	42
0.75 p.p.m.	6	Some apparent injury, no germination	35	Few appear injured	80
	24	All appear injured	0	All appear injured	Trace
1.00 p.p.m.	6	Trace of germination; some appear injured	46	Few " "	72
	24	All appear injured	0	All " "	Trace
1.50 p.p.m.	6	Few with short germ tubes; many appear injured	Trace	Few with short germ tubes; few appear injured	46
	24	All appear injured	0	All appear injured	Trace
2.00 p.p.m.	6	All appear injured	10	" " "	50
	24	" " "	0	" " "	0
3.00 p.p.m.	6	" " "	Trace	" " "	Trace
	24	" " "	0	" " "	0
Distilled H_2O	6	90 per cent budded	100	No germination	100
	24	100 per cent with short germ tubes	100	All have short germ tubes	100

Table 3 shows the effect of varying p.p.m. concentrations of soluble copper on the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*.

Copper concentrations approximating 0.50 p.p.m. were toxic to the conidia of *S. fructicola* in the same degree as saturated solutions of copper ethyl and butyl xanthates, while copper concentrations of 0.25 p.p.m. were comparable with methyl, propyl, and amyl xanthates. Nearly all of the copper xanthates are about as toxic to the conidia of *G. cingulata* as a solution of copper containing approximately 0.50 p.p.m. (about the copper solubility of weathered 4–4–50 Bordeaux mixture). The saturated solution of copper ethyl xanthate was the most potent, and the indication was that if the toxicity was due to soluble copper the copper content was nearer 0.75 p.p.m. than 0.50 p.p.m., since it was more toxic than the known solution of copper containing 0.50 p.p.m.

The data from the residue tests are shown in table 4. The 2 xanthates, copper ethyl and isoamyl, were each combined with hydrated lime and bentonite at the rate of 2 lb. of xanthate, 4 lb. of hydrated lime, 2 lb. of bentonite, and $\frac{2}{3}$ lb. of soluble fish oil soap to 100 gal. of water. Each mixture was sprayed on thin cover glasses and allowed to dry thoroughly. The sprayed cover glasses were then hung up among the leaves of unsprayed apple trees and allowed to weather. Samples were collected on the 3rd, 7th, 15th, and 22nd day following the initial exposure and brought into the laboratory for testing. Drops of conidial suspensions were placed on the residues and the preparations were held at a high humidity to prevent drying out and at a temperature favoring germination. Observations of the direct effect of the spray residues were recorded after a 24-hour period, and then some of the conidia were transferred to a medium free of residue, to find out whether the conidia had been killed or merely inhibited. Bordeaux mixture, the fungicidal value of which is known, was used for comparison.

Before weathering, the residues of the copper xanthates showed slight toxicity to the conidia of *Sclerotinia fructicola*, but none to those of *Glomerella cingulata*. Lime residues also exhibit toxicity under these conditions, and probably the first day's effect was due to the lime. As time elapsed, both of the xanthates showed a progressive increase in toxicity against the conidia of *S. fructicola* but none against those of *G. cingulata*. During the same period the progress of toxicity with Bordeaux mixture was more rapid, as is characteristic of that material. From these data it appears certain that the residues of the copper xanthate mixtures are not so potent as those of Bordeaux, but that they may have possibilities against disease-producing organisms that are killed by more dilute copper solutions.

Phytocidal tests on the bean (*Phaseolus vulgaris* L. var. Red Kidney) and on apple (*Malus sylvestris* Niell. var. Starking), under average greenhouse conditions of either low or high humidity indicated that the copper xanthates were relatively safe, since no injuries were observed.

Copper ethyl and iso-amyl xanthates, combined with lime, bentonite, and lead arsenate (when needed as an insecticide), and wetted with soluble fish oil soap, were applied in field tests to apple varieties in 1937 to control

TABLE 4.—*Effect of weathering on the fungicidal value of spray residues composed of copper xanthates, hydrated lime and bentonite*

Material	Days weathered	Total precipitation	Effect on *Sclerotina fructicola* conidia, 7 days old, grown at 75° F.	Subsequent growth		Effect on *Glomerella cingulata* conidia, 5 days old, grown at 75° F.	Subsequent growth	
				Treated	Check		Treated	Check
Copper ethyl xanthate mixture 2–4–2–100[a]	0	0	Inhibited—no growth	78	100	78 per cent germinated	100	100
	3	0	Some inhibited; some plasmolyzed	30	100	Inhibited—no growth	100	100
	7	0	Some inhibited; some plasmolyzed	32	100	" " "	100	100
	15	0.07	All appear plasmolyzed	Few	100	" " "	100	100
	22	0.07	" " "	0	100	" " "	100	100
Copper iso-amyl xanthate mixture 2–4–2–100[a]	0	0	Inhibited—no growth	82	100	68 per cent germinated	100	100
	3	0	Few appear injured; inhibited	62	100	All inhibited	100	100
	7	0	Some plasmolyzed; some inhibited	41	100	" "	100	100
	15	0.07	Many appear plasmolyzed; few inhibited	18	100	" "	100	100
	22	0.07	Mostly all plasmolyzed	6	100	" "	100	100
Bordeaux mixture 4–8–100[b]	0	0	All appear plasmolyzed	12	100	" "	100	100
	3	0	Mostly all appear plasmolyzed	Few	100	All appear injured or inhibited	46	100
	7	0	Mostly all appear injured	Few	100	All appear injured or inhibited	Few	100
	15	0.07	All appear plasmolyzed	0	100	All appear injured or inhibited	Few	100
	22	0.07	" " "	0	100	All appear injured or inhibited	Few	100

[a] Two lb. of xanthate, 4 lb. of hydrated lime, 2 lb. of bentonite, 100 gal. of water.
[b] Four lb. of copper sulphate, 8 lb. of hydrated lime, 100 gal. of water.

the apple scab organism, *Venturia inaequalis* (Cke.) Aderh. The data from these tests are shown in table 5. The tests were conducted on a small scale, on 5-year-old Williams' Early Red, York Imperial, Rome Beauty and Starking apple trees at the U. S. Horticultural Station, Beltsville, Md. In table 5 the performance of the xanthates is compared with that of phenothiazine mixture in one case, lime-sulphur followed by copper-phosphate mixture in two cases, and with no treatment in another.

TABLE 5.—*Effect of copper ethyl xanthate and copper isoamyl xanthate on the control of apple scab and russet**

Variety	Treatment	Total fruits	Scab		Russet	
			No.	*Per cent*	*No.*	*Per cent*
Williams' Early Red	Ethyl xanthate mixture[a]	240	6	2.5	18	7.5
	Isoamyl xanthate mixture	112	4	3.2	18	16.0
	Phenothiazine mixture[b]	334	2	Trace	40	12.0
York Imperial	Ethyl xanthate mixture	47	0	0.0	11	23.4
	Isoamyl xanthate mixture	27	0	0.0	3	11.1
	Lime-sulphur; copper-phosphate[c]	50	0	0.0	3	6.0
Rome Beauty	Ethyl xanthate mixture	11	0	0.0	4	36.4
	Isoamyl xanthate mixture	112	18	16.0	7	6.2
	Lime-sulphur; copper-phosphate mixture[c]	45	0	0.0	16	35.5
Starking	Ethyl xanthate mixture	228	10	4.3	35	15.4
	Isoamyl xanthate mixture	103	4	4.0	5	5.0
	No treatment	642	341	53.1	18	2.8

* Five applications: blossom, calyx, and 3 cover applications.

[a] Mixture = 2 lb. copper xanthate, 4 lb. of hydrated lime, 2 lb. of lead arsenate, and 2 lb. of bentonite to 100 gal. of spray fluid.

[b] " = 2 lb. phenothiazine, 4 lb. of hydrated lime, and 2 pounds of bentonite to 100 gal. of spray fluid.

[c] " = Lime-sulphur 1–40 at blossom, 1–50 at calyx, 1–75 at first and second cover applications. Copper phosphate 4 lb., hydrated lime 8 lb., bentonite 2 lb. to 100 gal. of spray fluid in third cover application. Lead arsenate 2–100 at calyx, first, second, and third covers.

The copper xanthates caused no injury that could be ascribed to their copper content. A type of injury developed, however, during the warm, humid periods, that greatly resembled that resulting from the use of arsenicals. On most varieties this injury affected mostly the margins of the leaves and caused but little damage. On the Williams' Early Red variety this injury also occurred in the intercostal regions of a few of the leaves.

Although the xanthates controlled apple scab fairly well under the circumstances of the tests, their performance, from a phytocidal and fungicidal standpoint, was inferior to that of the regular lime-sulphur and Bordeaux applications. They have no practical value as orchard spray materials at the present time.

SUMMARY AND CONCLUSIONS

The fungicidal value of the copper xanthates prepared from methyl, ethyl, propyl, butyl, and isoamyl alcohols were tested.

These materials are only slightly soluble in water. Saturated solutions contained very little available copper and apparently the copper and sulphur are combined so tightly that they are non-injurious to sprayed plants.

Copper ethyl and isoamyl xanthates were tested against the apple scab organism, *Venturia inaequalis*, in orchard experiments on 4 apple varieties. Although they reduced the number of scab infections, protection was not equal to that shown by the regular orchard treatments with lime-sulphur (early sprays) and copper phosphate mixture (late sprays).

In laboratory perfusion tests all of the xanthates tested proved toxic to some extent to the conidia of *Sclerotina fructicola*. The ethyl and butyl xanthates were the most toxic. None of the xanthates appeared to possess a high fungicidal value against the conidia of *Glomerella cingulata*. The toxicity exhibited by saturated solutions of the various xanthates against *S. fructicola* and *G. cingulata conidia* was of the same degree as that exhibited by a solution containing about 0.50 p.p.m. of copper.

Spray residues containing the ethyl and isoamyl xanthates were toxic to the conidia of *S. fructicola* but not to those of *G. cingulata*.

Applications of the xanthates on bean plants (*Phaseolus vulgaris* L. var. Red Kidney) and on apple (*Malus sylvestris* Niell. var. Starking), in the greenhouse caused no injury to leaves or stems at low and high humidities, respectively, and at average temperatures. In regular orchard treatments of apple varieties, copper ethyl and isoamyl xanthates appeared to promote arsenical injury when mixed with lead arsenate.

THE MECHANISM OF SPORE DISPERSAL IN PERONOSPORA TABACINA AND CERTAIN OTHER DOWNY MILDEW FUNGI[1]

J. A. PINCKARD[2]

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Sporangial release among the Peronosporales is usually described as a simple process brought about by the mechanical action of air currents or by rain. This concept assumes that the sporangia, upon reaching maturity, are broken off from their sterigmata-like attachments to the sporangiophore, more or less by accident. That this process is no mere accident was first suggested by de Bary (3), who wrote: "Where filiform sporophores rise free into the air, a further mechanical arrangement is found which greatly assists the shedding and scattering of the abscissed spores. It may be readily observed in the Hyphomycetes, in *Peronospora*, for example, *Phytophthora infestans*, and in the gonidiophores of *Peziza*, *Fuckeliana*, etc. The hyphae of these fungi are cylindrical in the moist and turgescent state, but collapse when dry *and especially* when the spores are ripe into a flat ribbon-like form, and the drier they are the more strongly do they become twisted round their own longitudinal axis. They are so highly hygroscopic that the slightest change in the humidity of the surrounding air, such, for instance, as may be caused by the breath of the observer, at once produces changes in their turgescence and torsion; the latter giving a twirling motion to the extremity of the gonidiophore and the ripe spores are thereby thrown off in every direction."

De Bary's interesting observations appear to have been either overlooked by most investigators or considered of little practical significance. Ingold (6), who reviewed the literature on spore discharge among land plants, quotes de Bary's statements in full, but adds: "It seems very likely that this kind of discharge may occur, but although I have examined the behavior of the conidiophores of *Peronospora parasitica* and *Botrytis cinerea* under conditions of rapidly changing humidity, I have been unable to convince myself that actual spore discharge is effected by the twirling movements of the conidiophore." Ingold concluded, therefore, that this form of spore discharge is of ". . . little or no biological importance. . . ." Weston (10), after studying exhaustively the production and dispersal of conidia in the Philippine *Sclerosporas* of maize stated, ". . . In the opinion of the writer these experiments and observations indicate that the conidia are forcibly thrown off from the sterigmata. . . ." Both de Bary and Weston, therefore, were of the opinion that mechanisms for forcible dispersal of sporangia occurred among certain Peronosporales.

[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist, Virginia Agricultural Experiment Station. The writer also wishes to express his gratitude to F. A. Wolf and to W. A. Whitney for helpful assistance.

During the course of extensive investigations (9) on the control of downy mildew of tobacco, caused by *Peronospora tabacina* Adam, the forcible ejection of sporangia of *P. tabacina* was observed to occur precisely as de Bary has described the process for *P. parasitica* (Pers.) Fr. The purpose of this report is to describe the hygroscopic mechanism of sporangial dispersal of *P. tabacina*, and similar fungi, and to discuss its importance in relation to the behavior of the downy mildew of tobacco (*Nicotiana tabacum* L.).

DEMONSTRATION OF THE HYGROSCOPIC MECHANISM

Several investigators (1, 11, 13) have described and illustrated the formation and production of the sporangia of *Peronospora tabacina*. Normally the sporangiophores and sporangia begin to form on the under surface of infected tobacco leaves a few hours before daybreak when temperature and atmospheric moisture are favorable. Numerous field observations (4) have shown that normal sporangial formation occurs within the range of 42° to 63° F., being most abundant at 56° F. Sparse sporangial formation will take place, however, both above and below these temperature limits (2). The effect of the water vapor pressure, or relative humidity, on sporangial formation, however, is pronounced. Armstrong and Sumner (2) have shown that sporulation is limited to the range from atmospheric saturation to slightly below the dew point. Weston (10), working with downy mildew of maize, observed that a film of moisture on the leaf seemed essential for normal sporulation. Sporangial formation by *P. tabacina* is inhibited, however, by the presence of a visible film of water on the leaf surface (Fig. 1).

If a leaf, bearing freshly-formed sporangiophores (Fig. 1), is mounted under a dissecting microscope, in such way as to show them in profile against a white background, and if the fruiting structures have not been subjected to drying between the time of maturation and examination, normal sporangiophores with their attached sporangia may be observed (Fig. 2, A). Then, if the normal, mature sporangiophores are watched, while slight drying begins, the entire crown of each fruiting structure, with its branchlets and sporangia, begins a counter-clockwise rotation. Each portion of a sporangiophore, lying between the branches, executes independent rotation. The degree of rotation appears to depend upon the length of the section between branches and the degree of desiccation.

Several complete twists occur in tall sporangiophores, in the portion extending up to the first branch. A lesser number of twists occurs between each successively shorter branch. As drying progresses, a twisting and bending motion is imparted to the sterigmata-like structure bearing the sporangia. If desiccation be discontinued, the movement ceases, and the rotation reverses itself, if air of a higher moisture content be supplied to the sporangiophores. Under conditions of delicate moisture balance, the breath of the observer is sufficient to bring about the above described movements. The net effect of these movements is, of course, the release of the

mature sporangia. Figure 2, A, B, and C illustrates diagrammatically the sequence of sporangiophore movements in an atmosphere becoming progressively drier. Figure 2, D illustrates the reverse movement of the sporangiophore shown in figure 2, C, after saturated air has been re-introduced.

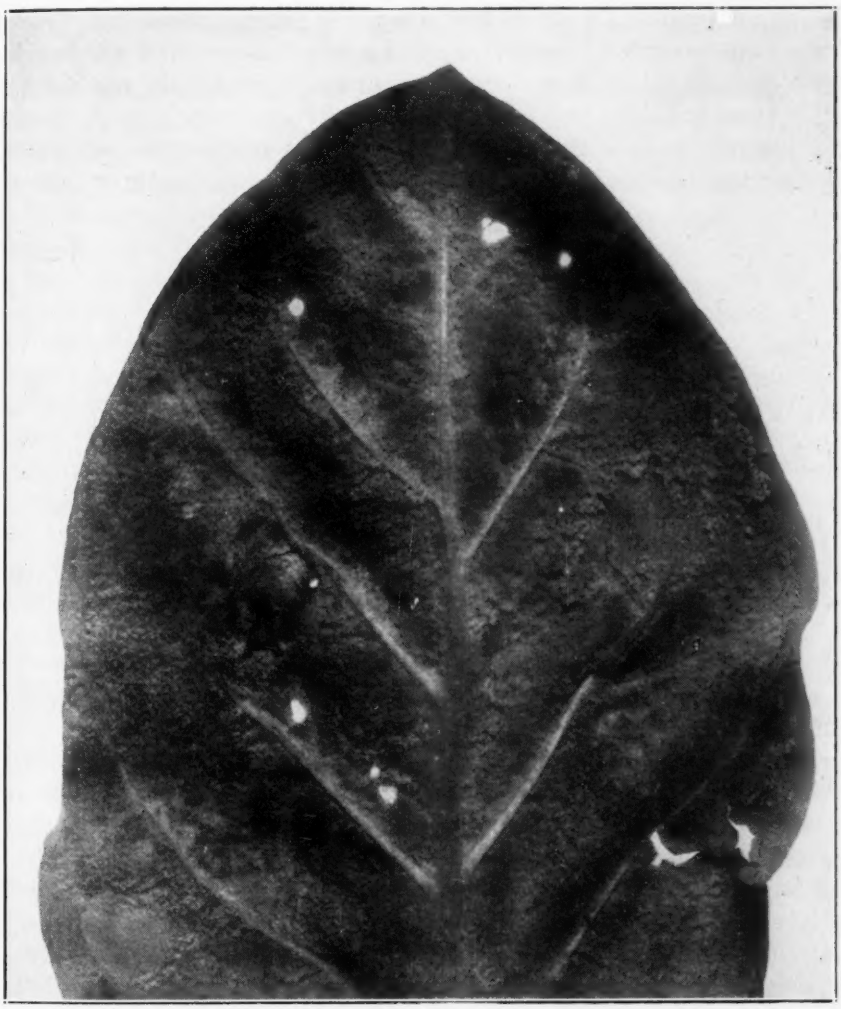

FIG. 1. Fruiting structures of *Peronospora tabacina* on the under side of a leaf of *Nicotiana tabacum*. A large drop of water prevented the formation of fruiting structures at point A.

During the course of rotation many of the sporangiophore branches become entangled with each other and the mature sporangia are dislodged from their attachments as a result of the spring-like action of the branches as they disengage.

The sporangiophores bearing immature sporangia are, however, sensitive

to air of varying moisture content and are capable of executing hygroscopic movement. Immature sporangia, however, are firmly attached, since none were observed to be released prematurely by branch entanglement following exposure to currents of dry air.

That the ejection of mature sporangia is not dependent upon entanglement was observed in the case of isolated sporangiophores mounted in a specially devised van Tieghem cell into which air was introduced through side arms at a very low velocity. Air at a constant temperature was bubbled slowly through sulphuric acid-water mixtures and led into the van Tieghem cell. As each bubble was introduced into the saturated atmosphere surrounding the isolated sporangiophores a minute decrease in water-vapor pressure was produced. As the experiment proceeded, the vapor pressure

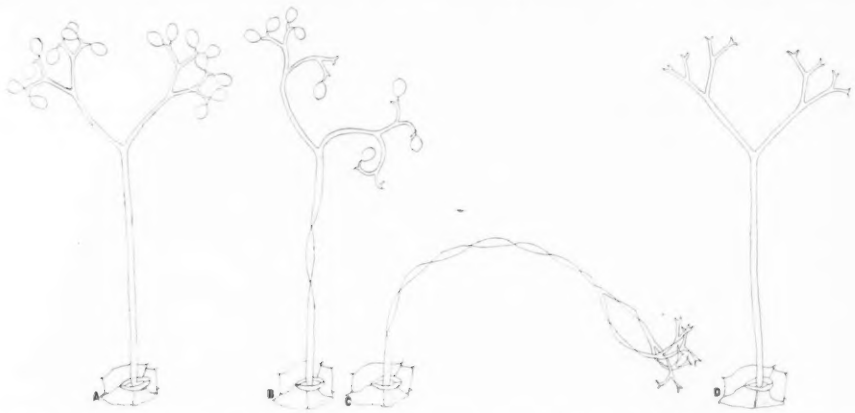

FIG. 2. The cycle of hygroscopic movement executed by a fruiting structure of *Peronospora tabacina*. A. Sporangia mature and attached, no significant vapor-pressure deficit. B. Slight vapor-pressure deficit; sporangial discharge begins. C. Strong vapor-pressure deficit; sporangia completely discharged. D. No signfiicant vapor-pressure deficit; sporangiophore returns to the normal position.

decreased gradually, and the sporangiophore rotated slowly. By alternately increasing the vapor pressure, branchlets bearing sporangia could be observed to rotate to the left or to the right. By slowly decreasing the vapor pressure, a point was reached when abscission occurred between the sterigma and its sporangium. The sporangium seemed to be released forcibly by energy applied at the point of attachment. The stimulus for this energy is attributed to differential stresses set up within the sterigmata.

If infected tobacco leaves are kept in a saturated atmosphere sporulation occurs but the sporangia are not released, since no hygroscopic movement takes place. Under such conditions the sporangia merely exhibit proliferation (13). If sporangia, freshly formed on leaves, are held in the saturated atmosphere of a closed container, and if a portion of such leaf is cautiously removed and exposed to "quiet" air during early morning, hygroscopic movements can be observed with a hand lens. The rotation varies from left to right, depending, presumably, upon the vapor pressure of the air striking the sporangiophores. In this connection, it was interesting to note

that, although the air may approximate saturation, erratic clockwise and counter-clockwise rotations of sporangiophores were observed. These movements were assumed to be sufficient to cause mechanical entanglement of sporangiophore branchlets and to effect the dislodgment of sporangia. It can be assumed, also, that mature sporangia were ejected by forces applied at their base.

SPORE DISPERSAL OF OTHER MEMBERS OF THE PERONOSPORALES

Other members of the genus *Peronospora* were examined, in the fresh state, to determine if they, too, exhibited hygroscopic mechanisms similar to those described for *Peronospora tabacina*. As a result, *P. parasitica* on *Lepidium virginicum* L., *P. geranii* Peck. on *Geranium carolinianum* L., and *P. halsteadii* Farl. on species of *Ambrosia* were observed to behave similarly.

In studying these downy mildews, it was necessary under certain conditions to mount the leaf bearing freshly formed fructifications under a prefocused microscope and to transport the leaf with its fruiting structures to the microscope, under very humid conditions. Collections were made before sunrise, and the examinations made immediately in a damp, semi-dark basement. After the material was in place on the microscope stage, a beam of light was focused upon it. After a few seconds, the heat from the light created a local vapor-pressure deficit in the field of vision and violent spore discharge was observed. Merely transferring leaves bearing sporangia from a moist chamber to the microscope stage, under ordinary laboratory conditions, caused mature sporangia to discharge. The delicate nature of the fructifications is correlated with a delicate balance of turgor pressure, which is easily upset by a vapor-pressure deficit when sporangia are mature and ready to abciss. This factor is evidently important in the development of field epidemics.

Dr. Charles J. Nusbaum, in a personal letter to the writer regarding the hygroscopic mechanisms of *Peronospora parasitica* on cabbage (*Brassica oleracea* L.) and *P. effusa* on lambs quarters, (*Chenopodium album* L.), stated, "In each case, the twisting appeared to be in a counter-clockwise direction under the microscope and was very pronounced. . . . The twisting of the main stalk and branches appeared to be nearly simultaneous and quite violent at the start. . . ."

DISCUSSION

The mechanism of sporangial discharge, described for *Peronospora tabacina*, contributes significantly to a better understanding of the life cycle of the parasite in relation to its environment. Numerous field observations over a period of several years have shown that sporangia are formed most abundantly during periods of approximate atmospheric saturation. These periods occur, in the field, during the early hours before sunrise. If saturation persists, the mature sporangia remain attached to the sporangiophore. Rainfall is, therefore, not a significant factor in causing dislodgment, as

many have heretofore believed. Dixon and associates (4, page 756) concluded after extensive field observations, that continued rainy weather, combined with long periods of saturation, although conducive to abundant sporulation, inhibits dissemination. Furthermore, Wolf and McLean (13) have shown that under conditions of saturation the mature sporangia merely proliferate *in situ*. These observations, together with those herein reported, seem to warrant the conclusion that downy mildew of tobacco is disseminated *following* atmospheric saturation and *not during* ordinary, continuous rain or during periods with no vapor-pressure deficit in the vicinity of the sporangia.

The period immediately following atmospheric saturation, or near-saturation, seems to be the best for dissemination of and infection by downy mildew fungi. During these periods conditions for sporangial germination are optimum. If sporangia were liberated during rains they would be washed from the air, and wide dissemination would seem impossible.

The combined action of air currents and vapor-pressure deficit cannot be separated successfully under field conditions, although it is possible to do so in the laboratory. Even though 100 per cent relative humidity prevails in the field, a slight rise in local air temperatures with the oncoming day will stimulate both sporangial discharge and air movement.

The mechanism of sporangial discharge seems to result from two nearly inseparable factors: mechanical entanglement of the sporangiophore branches, and the ejection of sporangia from their sterigmata by energy applied at the point of sporangial attachment. The exact nature of this latter process is yet to be determined; but, since the entire fruiting structure of *Peronospora tabacina* is developed in a few hours, and since its formation is dependent upon the presence of a nearly saturated surrounding atmosphere, it seems highly probable that the structure of the cell wall is not only very delicate, but also very sensitive to water vapor. Longitudinal thickening of the sporangiophore wall could account for differential stress during desiccation.

The fact that all sporangiophores rotated in unison, and in the same direction, suggests that, whatever the nature of the cell wall may be, it is probably heritable and a normal character of many species of *Peronospora*.

Weston (10) discusses the early literature dealing with the rate of fall of various air-borne spores and concludes that only a very slight air movement is sufficient to carry the Philippine Sclerosporas of maize significant distances. Wolf and associates (12) were able to trap from the air the sporangia of several downy mildews, including 4 of the 5 species discussed in this report. Newhall (8) was able to trap the spores of *Peronospora destructor* (Berk.) Caspary from the air over onion fields. Leach (7) and Doran (5) have, also, reported evidence that downy-mildew spores are wind-borne.

From the foregoing evidence it seems highly probable that the hygroscopic mechanism of *Peronospora tabacina*, *P. parasitica*, *P. geranii*, *P.*

halsteadii, and *P. effusa* has a definite function in the life history of these organisms. It also seems probable that other members of the genus have a similar mechanism for sporangial liberation. An understanding of these facts will undoubtedly lead to a better appreciation of the mode of spore dissemination and of disease development among the downy mildews.

SUMMARY

Sporangial dispersal, caused by changes in the moisture content of the air has been described for *Peronospora tabacina*, *P. parasitica*, *P. geranii*, *P. halsteadii*, and *P. effusa*.

Sporangial dispersal in these members of the Peronosporales begins with incipient drying, and is concluded by hygroscopic distortion of the aerial fructifications.

The mechanical action of wind and rain, during periods of atmospheric saturation, is not believed to be responsible for the dispersal of sporangia of these species of *Peronospora*.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE, MISSISSIPPI.

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COLD INJURY OF FLUE-CURED TOBACCO SEEDLINGS[1]

J. A. Pinckard and Luben Spasoff Bozovaisky[2]

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INTRODUCTION

A disorder of young tobacco seedlings, known locally as "white bud" or "yellow bud," has been observed in the bright-tobacco area of Virginia for the past several years. The disease has been noted only in the early spring about the time seedlings have a spread of $\frac{1}{4}$–2 inches.

The first symptom of this disorder (Figs. 1 and 2) is xanthosis of the young leaves enclosing the growing point. The result is a conspicuous and characteristic white or yellowish-white apical bud. As the small bud leaves expand, yellowing becomes evident along the margins but may disappear as the leaves grow older and no other noticeable symptoms develop. In more severe forms of the disorder necrosis of the marginal cells is a result. In such cases the young leaves may become permanently or temporarily distorted. If this happens, the plants usually suffer temporary retardation of growth. Thickened, glazed leaves of occasional seedlings usually are present in seed beds wherein this injury is manifest.

The cause of "white bud" in tobacco seedlings has been variously diagnosed as either too much or too little of one or another constituent of the seed-bed fertilizer. It also has been assumed to be an expression of frenching or of one of the numerous viruses attacking tobacco. Valleau and Johnson[3] have encountered this disease in Kentucky, and have correctly diagnosed it merely as an expression of cold injury.

The purpose of this paper is to describe experiments leading to the artificial reproduction of the above described cold injury of young tobacco seedlings.

MATERIALS AND METHODS

Seedlings of both the burley and flue-cured types of tobacco were grown in flats of a rich, loamy soil under strong illumination to encourage rapid, vigorous growth. When the plants were approximately $1\frac{1}{2}$ to 2 inches across, they were subjected to night temperatures of approximately 40° F., for several nights, for preliminary hardening. The night temperatures were then further reduced to 33° F., and below, for stated periods of time to study the symptoms induced by low temperatures upon the seedlings. Refrigerated plant-growing chambers, described elsewhere,[4] were employed for this purpose.

[1] Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Formerly Associate Plant Pathologist, and Senior Agricultural Scientific Aide, respectively, Virginia Agricultural Experiment Station. The writers also wish to express their gratitude to F. A. Wolf and W. A. Whitney for assisting in many ways.

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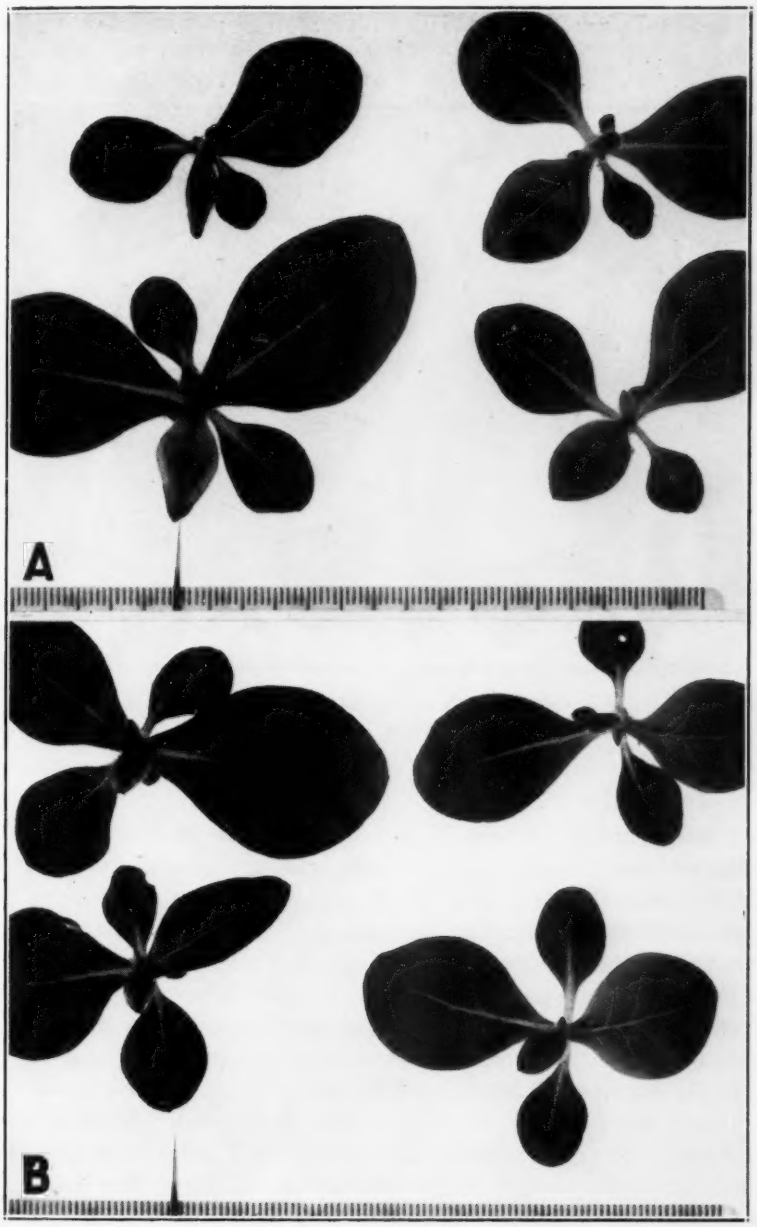

FIG. 1. Cold injury ("white bud") of flue-cured tobacco seedlings. A. Left, field-grown plants unprotected from cold during the night; right, plants protected by heavy muslin fumigation covers. B. Left, artificially-induced cold injury; right, untreated seedlings. Photographed approximately natural size, 5 days after exposure to cold. Contrast filter employed.

After subjecting the seedlings to various cold treatments they were again placed in a cool greenhouse, in strong illumination, and the effect of the cold treatment noted.

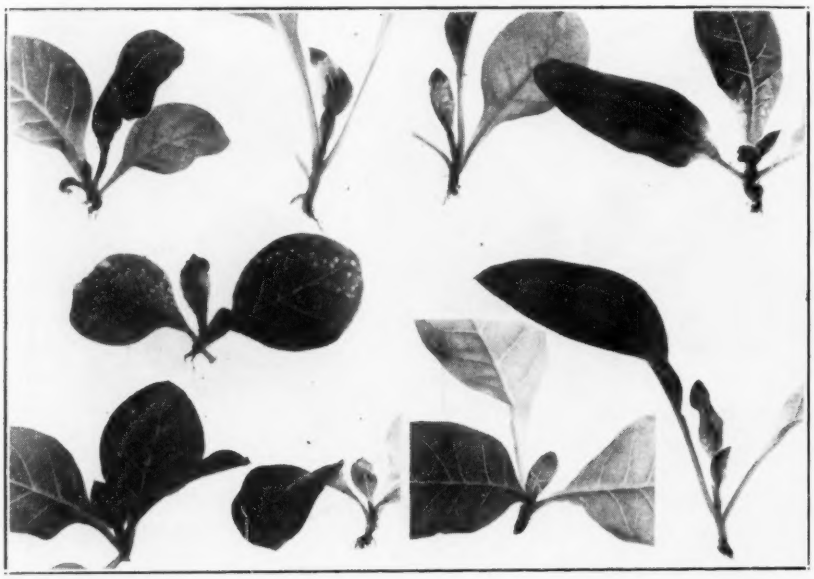

FIG. 2. Artificially-induced cold injury of burley tobacco seedlings brought about by air temperatures of 29 to 32° F. for a period of 16 hours. Photographed approximately 9 days after exposure.

RESULTS

Air temperatures between 31 and 33° F. for 15 hours sufficed to freeze both the soil and the leaves of seedlings growing in small flats. After the plants had thawed, wilting was observed; but the plants recovered without damage and were indistinguishable from untreated check plants.

Air temperatures between 29 and 32° F. for 16 hours were sufficient to cause the general cold-injury symptoms. Figure 2 shows these plants 9 days after treatment. Some plants in this series of experiments were not visibly affected by cold treatment; others exhibited various types and degrees of injury. If damage occurred, however, the young leaves enclosing the bud were always affected, and yellowing developed within a few days.

Air temperatures between 27 and 29° F. for 3 hours destroyed many of the buds outright, leaving the larger leaves unaffected and attached to a dead central axis. Several plants in this series of experiments showed glazed, thickened leaves, and the characteristic yellow apical bud leaves.

Air temperatures between 24 and 26° F. for $1\frac{1}{2}$ to $2\frac{3}{4}$ hours resulted in typical "white bud" symptoms (Fig. 1, B), similar to those of field-grown plants (Fig. 1, A). Thickened leaves with glazed surfaces appeared more abundantly at these temperatures than in any of the others. Both types of cold injury were quite apparent on the 5th day following cold treatment.

Field occurrence of the "white bud" type of cold injury was never observed in tobacco seed beds covered during cold nights with heavy muslin covers of the type used for fumigation and control of tobacco downy mildew. It did occur, however, following cold nights, in adjacent uncovered check seed beds. Figure 1, A, illustrates field-grown plants from adjacent uncovered and covered seed beds, while figure 1, B, shows a similar condition artificially induced.

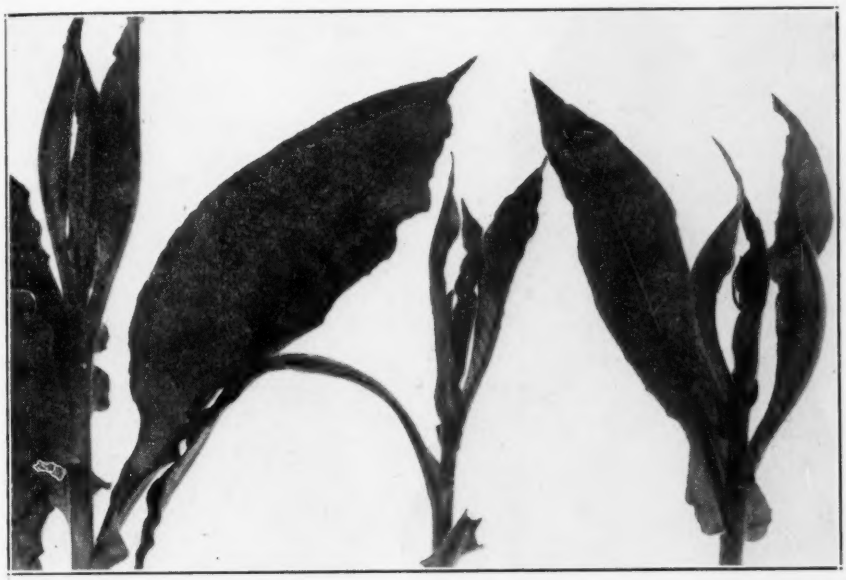

FIG. 3. Natural frost injury of old tobacco plants showing typical sensitivity of immature tissues. Reduced.

Cold injury, as a result of frost, on old, field-grown plants (Figs. 3 and 4) appeared to be similar in many respects to cold injury to seedlings. The young marginal leaf tissue and the growing point, as in the case of seedlings, were most susceptible to cold, while peculiar patterns and blanching developed on old leaves.

DISCUSSION

Tobacco seed beds in Virginia usually are prepared and sown during the first period of warm weather in January. The seeds lie dormant in or on the ground until the warm weather of spring brings about germination and subsequent growth. Late snowfall or freezing weather may follow warm periods after plants have emerged and relatively large leaves have developed. Depending upon local conditions, ice may form on the leaves without causing significant damage.

Experiments reported in this paper indicate that both the leaves of prehardened tobacco seedlings and the soil supporting them may become solid from ice crystals for several hours, without causing significant damage to the plants. It appears, however, that if temperatures drop below 30 or 31°

F. for a few hours damage may result. As the temperature decreases, as may be expected, the injury increases. The first symptoms of injury appear to be a blanching of the primordial tissues, which results in the symptoms commonly observed as "white bud." Plants showing such symptoms may

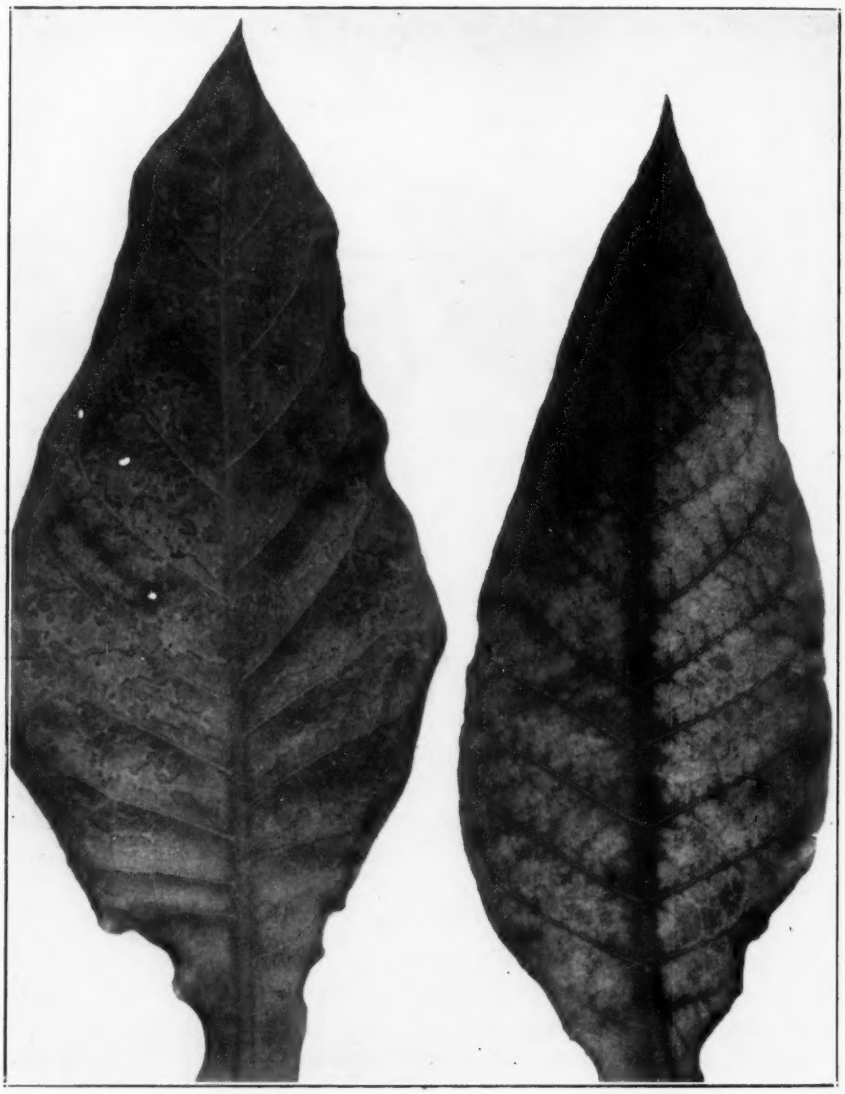

FIG. 4. Natural frost injury of old tobacco leaves showing peculiar patterns and blanching. Reduced.

have been exposed to temperatures as low as 24 to 26° F. but for not more than 2 or 3 hours.

As cold injury became more severe, the marginal tissues of very young leaves were destroyed progressively toward the base. The central axis of the

plant and the growing point were destroyed before older leaf tissue was harmed. In older plants similar conditions were encountered, except that on old leaves irregular color patterns and blanching appeared.

SUMMARY

Incipient cold injury to tobacco seedlings, of both the burley and flue-cured types, resulted in bleaching of the bud leaves. This disorder, common in early spring, appears to be induced by an exposure of 2 or 3 hours at temperatures between 24 and 26° F., or in 15 to 16 hours at temperatures between 29 and 32° F. Typical symptoms of the so-called "white bud" disease, a phase of cold injury, appeared 4 or 5 days after plants were exposed to these temperatures.

The first tissues in tobacco leaves to succumb to cold were those at the margins of very young leaves. This appeared to be true of both seedlings and older plants. More intense cold destroyed the apical meristem in both young and old plants, while cold injury to mature leaves resulted in irregular patterns and blanching.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE, MISSISSIPPI, AND
VIRGINIA AGRICULTURAL EXPERIMENT STATION,
TOBACCO RESEARCH LABORATORY,
CHATHAM, VIRGINIA.

A SEED-BORNE MOSAIC OF ASPARAGUS BEAN, VIGNA SESQUIPEDALIS

W. C. SNYDER

(Accepted for publication August 27, 1941)

During a period of 1938 when frequent greenhouse plantings of asparagus bean, *Vigna sesquipedalis* Wight, were being made, it was observed that a small percentage of the plants developed mosaic symptoms. The seed involved was from a commercial lot purchased under the name of Yardlong Bean. This paper is concerned with the description of this disease and the determination of its nature.

SYMPTOMS

The symptoms of asparagus bean mosaic (Figs. 1, 2) are marked by a light and dark green mosaic, frequently attended by a downward rolling of the leaflets. Leaf rolling is often pronounced. The dark-green areas tend to become puffy and result in mild rugose symptoms, or leaf distortion (Fig. 2, C and D). Variations in the mosaic pattern occur, as they do in the case of common bean (*Phaseolus vulgaris* L.) mosaic caused by *Bean virus 1* Pierce. Frequently the dark-green areas form broad bands along the main veins of the leaflet, as seen in figure 1, the remainder of the leaf being lighter green. Occasionally the primary leaves of plants infected through seed transmission of the virus show mild mosaic symptoms or slight leaf distortion and stunting (Fig. 2, A). Infected plants often are dwarfed, especially if infection has taken place through the seed, and growth may be less robust than in normal plants. Severely affected plants frequently set little or no seed.

JUICE TRANSMISSION

Juice expressed from diseased plants was used to inoculate healthy plants obtained from a stock of disease-free seed, employing the rubbing method of inoculation aided by the use of carborundum as an abrasive. The infection of over a hundred healthy plants was obtained at the outset of the work in this way with the production of the characteristic mosaic symptoms shown in figures 1 and 2. Plants held as controls remained different tests, ranging from less than 10 per cent to more than 90 per cent. healthy.

Usually 9 to 28 days elapsed from time of inoculation until appearance of symptoms, apparently depending upon conditions prevailing in the greenhouse. The percentage of infection obtained varied considerably in the

APHID TRANSMISSION

The pea aphid, *Macrosiphum pisi* (Kalt.), was used to test the insect transmissibility of the virus. Noninfected aphids were reared on plants

of *Vicia faba* L. grown under cages. Colonies were transferred to mosaic-infected plants of asparagus bean, where they were allowed to feed 2 days before being transferred to healthy plants in numbers of 10 to 25 aphids per plant. After 2 days the aphids were removed by thoroughly spraying the plants with nicotine sulphate solution. Controls consisted of healthy asparagus bean plants on which like numbers of noninfective aphids were

FIG. 1. Vein-banding symptoms of asparagus bean mosaic.

placed. Of 14 plants on which infective aphids had been allowed to feed, 10 became infected with mosaic. No mosaic developed in 9 plants on which noninfective aphids were fed. These results sufficed to demonstrate that the virus belongs to the group of aphid-transmitted viruses, and no further tests with aphids were made. It seems probable that other species of aphids which feed naturally on asparagus bean also may be vectors of the virus,

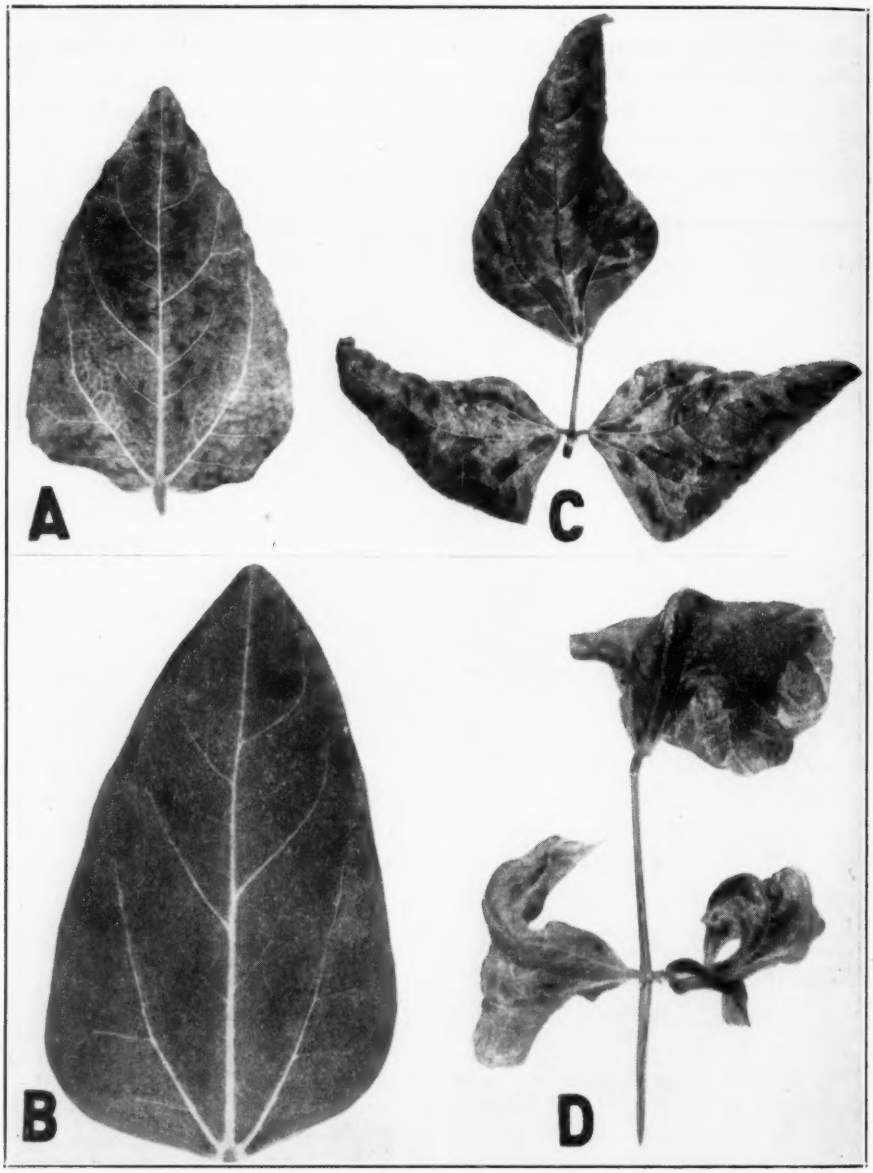

FIG. 2. A, first symptoms of asparagus bean mosaic on a primary leaf infected through the seed; B, healthy primary leaf of the same age as in A; C, rugose mosaic, and D, severe leaf distortion symptoms of asparagus bean mosaic on trifoliate leaves.

judging from the experience gained from studies of other aphid-transmitted legume viruses.

SEED TRANSMISSION

It has already been mentioned that the appearance of this mosaic of asparagus bean was first seen in plantings from a certain lot of commercial

seed grown in a greenhouse. In a count made of infected and healthy plants from a planting of this seed lot, 4, or 3.3 per cent, were infected out of a total of 121 plants.

A few infected plants were allowed to mature in the greenhouse, and the seed from them replanted. Of 109 plants grown from the seed set by diseased plants, 41, or about 37 per cent, developed mosaic symptoms. These figures illustrate a seed transmissibility of the virus resembling that of the well-known common bean-mosaic virus. The seed-borne nature of the virus serves to distinguish it from most other viruses, as well as to stress its similarity to those of common bean mosaic, cowpea (*Vigna sinensis* Endl.) mosaic, and soybean (*Soja max* Piper) mosaic (Soybean virus 1 Pierce).

HOST RANGE

Attempts to infect other plants, particularly legumes, have mostly failed. Infection of cowpea has been obtained, with symptoms resembling those of cowpea mosaic, but only in certain instances has the infection of common bean (*Phaseolus vulgaris*) been successful. In the latter case symptoms have not agreed with those of common bean mosaic. Plants inoculated, but that so far have not shown infection, include catjang (*V. catjang* Walp.), Midwest soybean (*Soja max*), Perfection and Laxtons Progress pea (*Pisum sativum* L.), common and Peruvian alfalfa (*Medicago sativa* L.), *Acer arietinum* L., *Lathyrus odoratus* L., *Melilotus alba* Desr., *M. indica* All., *Trifolium repens* L., *T. pratense* L., *Nicotiana tabacum* L., and *N. glutinosa* L.

Likewise, an attempt to infect the asparagus bean with the common bean-mosaic virus was without results. It would appear from these tests on host range, although not exhaustive, that the virus causing this mosaic of asparagus bean is narrowly limited in its infectivity, and that it is probably largely confined in nature to the genus *Vigna*.

PROPERTIES OF THE VIRUS

The thermal inactivation point, dilution end point, and longevity of the virus were determined in the usual way. The expressed juice from infected plants was strained through cheesecloth, treated, and then inoculated into healthy asparagus beans by the rubbing method. The results obtained are shown in table 1.

TABLE 1.—*Physical properties of the asparagus bean-mosaic virus*

Thermal inactivation			Dilution tolerance			Longevity *in vitro*		
Temp.	No. plants inoculated	No. plants infected	Dilution	No. plants inoculated	No. plants infected	Days	No. plants inoculated	No. plants infected
Room	35	17	None	33	21	None	34	21
55° C.	37	15	1/1000	30	2	1	41	9
60° C.	34	0	1/3000	27	0	2	24	1
65° C.	32	0	1/6000	21	0	4	23	0

These results (Table 1) show that the virus in juice extracted from diseased plants was inactivated by heating it for 10 minutes at a temperature of 60° C.; by diluting it to a concentration of 1–3000; and by keeping the juice in stoppered test tubes for about 2 days at a constant temperature of 21° C.

DISCUSSION

Mosaic diseases of asparagus bean, cowpea, and catjang were observed in field plots in Indiana by Gardner (1) in 1925. No attempt was made to study the viruses causing these diseases other than to demonstrate that the cowpea mosaic was seed-borne (2, 3).

Nelson (4) cites the asparagus bean as a host of common bean mosaic virus, but others, including Pierce (5), and Zaumeyer and Wade (6), were unable to confirm Nelson's findings. The recent studies of McLean (8) indicate that the cowpea-mosaic virus may cause a mosaic of catjang, but, apparently, the asparagus bean was not tested. That the asparagus bean virus may differ from the cowpea virus is suggested in the present paper by the failure of the former to infect catjang, and by the difference between the thermal inactivation points of the two viruses (McLean reports 72° to 75° C. for the cowpea virus). In addition, the symptoms produced on cowpea have not always been the same as those described for cowpea mosaic. These distinctions are not large, and they suggest that closely related viruses or perhaps even strains of one virus may be involved in the two diseases.

On the bases of symptoms, seed carriage, aphid transmissibility, and properties, the asparagus bean mosaic clearly belongs to the group of viruses that cause, respectively, the seed-borne mosaics of common bean (9), soybean (7) and cowpea (3). Each of these viruses appears to be restricted in nature to one or to a few species, and this also appears to be true in the case of the asparagus-bean virus. Here is an instance where several apparently distinct virus diseases show a remarkably close relationship in respect to cause. In a biologic sense these viruses might well be considered to be physiologic forms of the same virus species. They show similar thermal inactivation points (McLean's results on the cowpea virus place it somewhat out of line with the other viruses in respect to the thermal inactivation point), longevities *in vitro*, dilution tolerance, seed transmissibility, aphid and mechanical transmissibility, and limited host ranges, as well as a similarity in symptoms. Essentially then, these viruses differ primarily in their specialization as to host, and might be considered as variants of a common virus, which have become more or less adapted to specific hosts.

SUMMARY

A mosaic disease of asparagus bean, *Vigna sesquipedalis*, recovered from a lot of commercial seed, is described.

The causal virus was transmitted from diseased to healthy plants both by the mechanical inoculation of expressed juice and by the pea aphid.

The virus is seed-borne, and in this respect it closely resembles the viruses that cause common bean mosaic, cowpea mosaic, and soybean mosaic.

Properties of the virus are shown to be as follows: thermal inactivation point, between 55° and 60° C.; longevity *in vitro*, about 2 days; and dilution end point, nearer 1/1000 than 1/3000.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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INJURY TO TOMATO SEED IN DISINFECTION

J. H. Miller and Raymond Grogan

(Accepted for publication September 6, 1941)

While making routine tests for germination and contamination of treated tomato seed, the senior writer has frequently received preliminary samples from commercial houses that showed a very marked loss of viability. Then, later examinations of samples from the entire lot treated in bulk often failed to show the low germination of the first samples. The method of treating was the same in both cases, so it seemed possible that there might be an important factor in the ratio between the quantity of seed and the volume of solution.

The object of this investigation then was to determine the effect on germination of varying the proportion of seed to solution. The relation of this varying ratio to the residual mercury content of the solution will be considered in a later paper.

MATERIALS AND METHODS

This experiment was divided into the following parts:

1. The quantity of seed was constant at 10 g., and the volume of solution was varied from 10 cc. to that amount of seed, to 100 cc. with 10-cc. intervals, and then continued up to 500 cc. with 100-cc. intervals. Germination tests were run after each trial.

2. The volume of solution was constant at 100 cc., and the amount of seed was varied from 1.5 g. up to 48 g. The intervals were secured by doubling the quantity of seed each time. Enough classes were placed in this experiment to check the high points of the first. This test reverses the first in that it begins with a wide ratio and ends with a narrow one. Germination tests followed as above.

3. Disinfection tests were made from each trial by plating out 200 seeds on dextrose-potato agar in Petri dishes and counting the number showing contaminations on the fourth day. This was for the purpose of determining if there is a relationship between the effectiveness of treatment and the varying seed-solution ratios.

The seed used in this investigation was Stark's Earliana variety from the 1940 crop. These were nontreated, and handled as ordinary commercial seed.

Two standard solutions were used for treating.

1. Mercuric chloride, C.P., at rate of 1 g. to 3000 cc. of water. Seeds were stirred constantly while in the solution, and aften ten minutes they were taken out and rinsed in three changes of distilled water, and then dried on paper towels.

2. Ethyl mercury phosphate, 5 per cent (New Improved Ceresan), at rate of 1 g. to 1200 cc. of water. These were treated as above for 10 min.,

but were dried without rinsing. The concentrations remained constant in all trials.

The germination tests were made in a standard hot-water-jacket germinator. Two hundred tomato seeds from each different solution were placed on blotters and counts were made at 4, 7, and 11 days, respectively.

The entire experiment was executed 3 times, and the curves shown in the accompanying graphs represent the average of the three.

EXPERIMENTAL RESULTS

There were no material changes in the percentage germination at the end of the 4th day (Fig. 1) until a ratio of 1 part of seed to 6 of solution had been reached for the Ceresan and 1 to 5 for the mercuric chloride. After this both rather consistently caused a decreased germination, and at 1 to 50 only 1.15 per cent germinated for Ceresan and 0.83 per cent for mercuric chloride. The seed treated in the latter solution showed a lower germination in all solutions, except the 1 to 7 and 1 to 9, when the two were about equal.

The curve of the 7th day (Fig. 1) also shows a decreased germination with the widening ratios after 1 to 6 for Ceresan and 1 to 8 for mercuric chloride. The 1 to 50 ratio caused a decline to 7.98 per cent for Ceresan and 2.33 per cent for mercuric chloride.

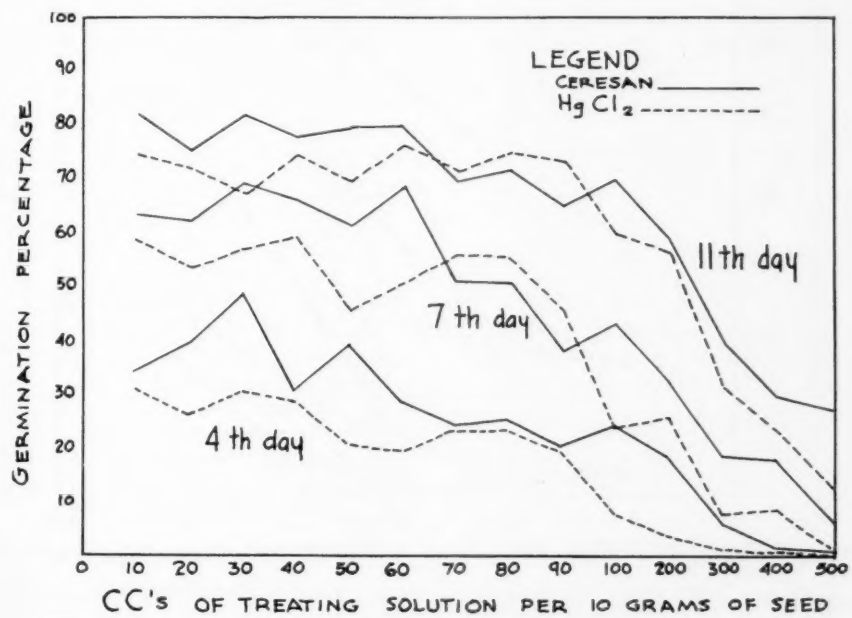

FIG. 1. Curves showing the effect on germination of varying the ratio between the volume of treating solution and the quantity of seed.

The final germination counts were made on the 11th day and then (Fig. 1), there was a significant decline in the gradient following a solution of 1

to 6 for Ceresan and 1 to 9 for mercuric chloride. When 1 part of seed was placed in 50 cc. of solution the final germination was 27.74 per cent for Ceresan and 13.0 per cent for mercuric chloride.

In the second experiment, where the volume of solution was maintained a constant and the quantity of seed was varied the curves (Fig. 2) are reversed. The wide ratio of 1.5 parts of seed to 100 cc. of solution resulted in 1.3 per cent for the 4th day, 3.9 per cent for the 7th and 17.1 per cent for the 10th day for Ceresan, and 2.75 per cent, 17.17 per cent, and 34.3 per cent, respectively, for mercuric chloride. On the other hand, the narrow ratio of 48 to 100 produced a germination of 25.8 per cent, 53.8 per cent and 72 per cent for the former treatment and 25.1 per cent, 61.9 per cent, and 78 per cent for the latter.

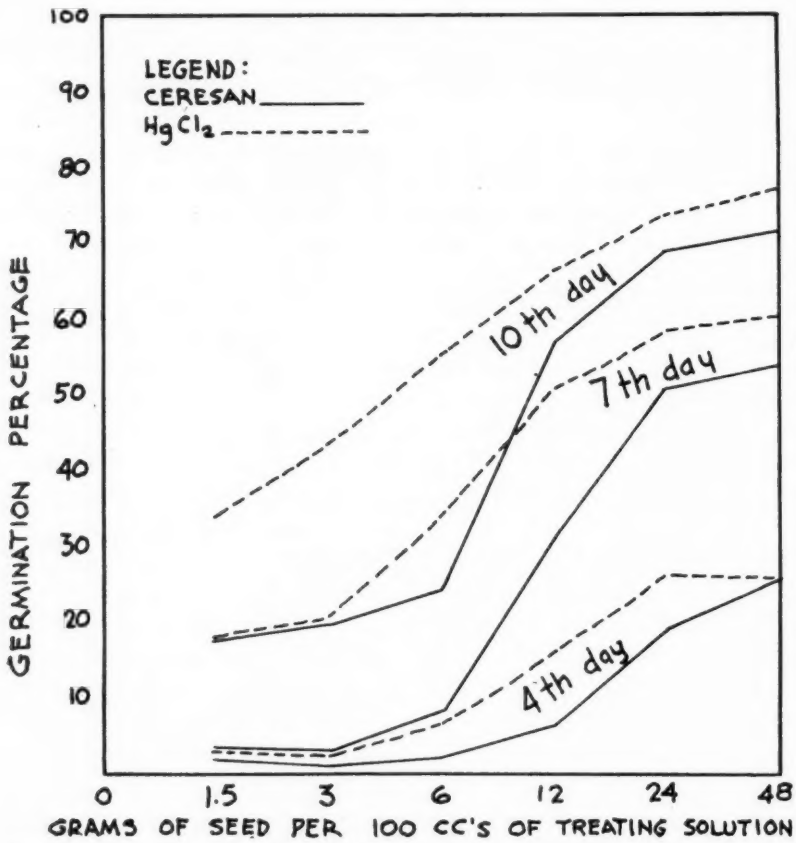

FIG. 2. Curves also showing the effect on germination of different seed-solution ratios with the variable being the quantity of seed.

Both experiments show the same material decrease in seed germination following a widening of the ratio, with a critical point at about 1 of seed to 7 to 9 parts of solution.

CONTAMINATION STUDIES

In seed treatment the two chief points to be considered are effectiveness and seed injury. The following table gives the results of plating out 200 seeds from each treating trial.

TABLE 1.—*The effect on seed contamination of varying the volume of solution*

Seed	Solution	Ceresan contamination	Mercuric chloride contamination
g.	*cc.*	*Per cent*	*Per cent*
10	10	41.8	20.0
10	20	5.8	1.8
10	30	3.3	0.0
10	40	1.8	0.25
10	50	0.5	1.1
10	60	0.0	0.0
10	70	0.0	0.0
10	80	0.0	0.0
10	90	0.0	0.0
10	100	0.0	0.0

No organisms grew from the seed after treatment of 1 to 6.

TABLE 2.—*The effect on contamination of varying the quantity of seed*

Seed	Solution	Ceresan contamination	Mercuric chloride contamination
g.	*cc.*	*Per cent*	*Per cent*
1.5	100	0.0	0.0
3.0	100	0.0	0.0
6.0	100	0.0	0.0
12.0	100	0.5	6.0
24.0	100	0.0	9.0
48.0	100	3.0	12.5

This second table was obtained by plating out seeds from the germination trials of the second experiment. This shows that no organisms appeared beyond a proportion of 12 to 100, or 1 to $8\frac{1}{2}$. The wider ratios produced complete disinfection of the seed.

The Ceresan solutions in the contamination studies (Table 1) did not produce as efficient disinfection as mercuric chloride up to 1 to 4 ratio, and in the corresponding germination graph (Fig. 1) the reduction in germination is also not so great. Then in table 2 the reverse is shown; that is, the Ceresan is ahead of the mercuric in disinfection and the graph (Fig. 2) shows a greater decrease in germination. It is, therefore, evident from this study that as the treating solution becomes more efficient there is a resulting sacrifice of seed viability. This, however, is not a general rule, as past laboratory experience has shown that heavily infected seed often germinates better after treatment.

SUMMARY

The experimental evidence indicates that when the ratio between quantity of seed and volume of treating solution is increased above 1 to 8 the germination of tomato seed is progressively impaired.

The curves for Ceresan and mercuric chloride are not significantly separated in figure 1, and in figure 2 the trends are reversed. So one cannot logically predict a difference in germination depression between the two from the results of this investigation. Both of them, however, show the same downward trend of the curves in wide ratios.

The ratio of 1 to 8 produced complete disinfection and did not cause a too serious drop in germination.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA.

GROWING NEW ROOT SYSTEMS BY SOIL BANKING—A PROMISING METHOD OF REJUVENATING TREES ATTACKED BY ROOT DISEASES

ARTHUR S. RHOADS[1]

(Accepted for publication September 9, 1941)

Although the life histories of many of the organisms causing root rot of woody plants have long been understood, little progress has been made in the control of these diseases. As a whole, this group of diseases has received much less attention by plant pathologists than most other groups, and certainly far less than their economic importance merits. In the case of the mushroom or toadstool root-rot fungi, it is universally conceded that it is difficult to adopt effective control measures and that if trees become attacked their treatment is tedious and expensive and the results uncertain. However, varying degrees of success have been reported by a number of investigators.

The methods that have been used most widely thus far in the treatment of root diseases of woody plants have been confined largely to (a) surgical treatment, involving excision of all diseased tissues and dead roots, followed by painting the exposed wood surfaces with various paints, or even all the exposed roots with various disinfectants; (b) the aeration method in which the soil is removed from under the base of the attacked tree and the adjacent main roots to check the development of the causal organism by desiccation; (c) application to the soil of various chemicals in an effort to arrest the development of the causal organism or eradicate it, preferably without sacrificing the attacked tree; and (d) the isolation method, accomplished by digging of trenches or erection of barriers around infected areas to prevent further spread of the fungus through the soil. Various combinations of these methods have, of course, been employed.

The first two of the above methods have proved fairly effective, especially when combined, if applied before a large percentage of the roots are killed. The third has proved ineffective so far as saving attacked trees is concerned, though, if one is prepared to sacrifice the attacked trees and sometimes also adjacent unattacked ones, injection of carbon bisulphide in a network of holes in the soil has proved an effective means of eradication, at least in light, porous, fairly dry soils and under favorable temperature conditions. Even with this method, because of difficulty of eradicating the mycelium within the interior of large roots, it is recommended that such roots be pulled or dug out before treating the soil. The last method, which was suggested for combatting the spread of *Armillaria mellea* before it was found possible to eradicate the fungus by soil disinfection, appears impractical.

This paper is designed to direct attention to the value of another promis-

[1] Formerly Plant Pathologist of the Florida Agricultural Experiment Station, in charge of the Citrus Field Laboratory at Cocoa.

ing method that appears to be not widely known or appreciated, though it has been used in Florida for several years. It consists in banking soil around the bases of attacked trees to stimulate development of new roots. The readiness with which citrus trees and many other trees and shrubs develop adventitious roots when the soil is worked up above the normal ground level has suggested the possibility of utilizing this natural tendency to develop adventitious roots as a means of growing new root systems on trees whose life is threatened by various root diseases. Instances have been observed frequently where citrus trees, killed back in the freezes of 1894–5, have developed roots in the interiors of the hollow shells left by the rotting away of the central wood of the original trunks, in the litter accumulated therein. Indications of new root development are seen frequently also on the exterior of citrus trees, where a callus has formed following some basal injury and the soil has been worked up somewhat above the usual level. Several instances have been noted in Florida citrus groves where the soil was allowed to remain in place for a few years after banking for cold protection during the winter season. In such instances, when the banks were torn down subsequently, roots as large as a man's arm were found to have developed, giving the trees a secondary system of brace-roots similar to a red mangrove (*Rhizophora mangle*).

In 1924, and subsequently, Jeffries (3, 4, 5) reported a method of giving citrus trees new root systems through partial ringing, followed by banking. This method was discovered following the usual practice of banking, in the fall to guard against freezing, some young trees, which had been partly girdled to force bud growth. When the banks were removed at the end of the winter season a splendid new root system was found to have developed from the margin of the callus formed immediately above the girdle. He found this method desirable for giving trees a new root system when budded to an undesirable rootstock, and preferable to inarching. He also suggested the applicability of this method for saving sweet seedling orange trees attacked by foot rot.

In 1926, Höstermann (1) reported that *Caragana arborescens* and *Laburnum vulgare* could be propagated successfully by ringing followed by mounding with soil. Shoots of both plants ringed in August developed extensive root systems by November from the callus formed above the wounds. He later (2) extended these experiments to 43 species of fruit stocks, fruit varieties, nuts, ornamentals, etc. Compared with ringing, a tight wire band gave more favorable results with respect to callus formation and rooting, making possible the vegetative propagation of 31 of the 43 species.

In 1926, the writer (6) briefly reported the successful rejuvenation of old citrus trees, nearly dead from basal girdling by foot rot (*Phytophthora parasitica*), which had been accomplished successfully by a grower at Umatilla, Florida, by the simple expedient of merely banking soil around the bases of the trees without any preliminary treatment. This experiment

was begun on a number of old, half-dead seedling orange and grapefruit trees in 1920, and was subsequently extended to other trees after its success became apparent. In the initial trees treated, which were first examined in 1925, a rough framework was built about the base of each tree treated and this was filled with two wagon-loads of clay, covering the base of the trunk to a height of from 15–18 inches. The framework was used to better retain the soil about the bases. Sand has been used for this purpose in various parts of the State, but is much more subject to washing away when no framework is provided and is less retentive of moisture. In from 2–5 years

FIG. 1. New roots produced by banking old seedling grapefruit tree nearly dead from foot rot, showing how roots developed from margin of callus above old decayed areas have bridged the girdle and effected rejuvenation of tree.

a more or less profuse growth of new roots developed from the margin of the callus formed above the bases of the partly girdled trunks. In those cases where the soil was washed away for examination about 2 years after banking, good-sized roots were found bridging basal lesions where the wood had become extensively decayed (Fig. 1). In all cases the trees no longer exhibited the thin, yellow tops characterizing trees attacked by foot rot, but, following the establishment of the new root systems (Fig. 2), had developed healthy tops with good fruit production. The trees thus treated in this grove still continue to thrive. This simple treatment has added many years

of productivity to old, declining trees that were so badly girdled and rotted at the bases that they would soon have died or blown over. This method of treatment has been recommended in Florida for the past 15 years, especially as a last resort for trees that have declined too far to save by the usual methods of treatment.

The stimulation of the production of a new root system also has been found readily applicable to the so-called Australian pines (*Casuarina* spp.) attacked by Clitocybe root rot (*Clitocybe tabescens*). This disease works so insidiously in the *Casuarinas* that trees are often extensively girdled by the time a slight yellowing of the foliage branches becomes apparent on the

FIG. 2. Rejuvenation resulting from banking old seedling grapefruit tree nearly dead from foot rot, showing splendid development of new roots after partial washing away of bank.

lower limbs, which is the first symptom to manifest itself in the tops of the trees.

The banking method of treatment was first tried on a tree of *Casuarina lepidophloia* that had been kept formally pruned to a cylindric shape, being one of several such trees in the yard of the writer's residence at Cocoa, Florida. When clipping this tree at the end of December, 1932, it was noticed that the lower limbs exhibited a slight pallor of the foliage branches and, at the base, a cluster of recently dried mushrooms of *Clitocybe tabescens* was found. Upon removing the soil to investigate the root system with a view to attempting to save the tree by surgical treatment, all the roots but three small lateral ones, of finger-size, on one side were found dead and invaded by the mycelium of this root-rot fungus. It was necessary to cut

off and remove all the lateral roots but these, the base of the tree with the divisions of the taproot, and the basal bark, where the fungus had completely girdled the tree. The girdling even involved the 3 small lateral roots at the point where they left the root crown. All diseased bark was cut out down to the wood and the exposed wood surfaces were painted with a mixture of roofing paint and carbolineum. The tree was propped to hold it in position, but was blown to the ground the day following the treatment and had to be guyed into position with wire.

It was realized that this tree could not survive after this drastic treatment without further help. It was thought that if a new root system could

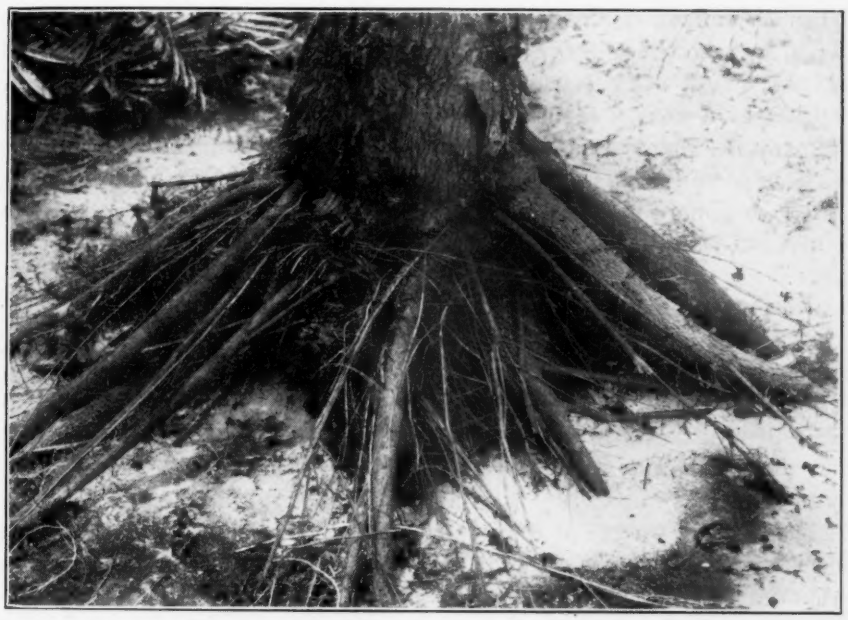

FIG. 3. New root system developed 4½ years after surgical treatment and banking of Australian pine (*Casuarina lepidophloia*) so nearly dead from Clitocybe root rot that all roots except three small lateral ones of finger-size on one side were removed and guying was required to hold tree in position.

be induced to develop from the margin of the living bark above the completely girdled base before the 3 remaining small girdled lateral roots ceased to conduct sufficient water to keep the tree alive there might be a remote possibility of saving it. A well of concrete blocks about a yard square was laid around the tree, filled with sandy soil, and watered. Following a prolonged drought, this tree lost a large proportion of its foliage branches in June, 1933, and looked as though it were going to die. However, occasional watering enabled it to survive, and, following the development of new roots, it began to improve after several months. By the middle of 1934 these new roots had so developed that the tree was as firmly anchored as it was originally. The well of blocks, therefore, was removed.

When clipping this tree again in late April, 1937, the foliage branches on the lower limbs were observed to have become distinctly yellow again; investigation showed that the tree had become two-thirds girdled by Clitocybe root rot. The remaining mound of sand was washed away with a hose on May 16, and figure 3 shows the luxuriant development of new roots at this time.

The tree was re-treated by cutting away all diseased areas of bark on the base of the trunk and more than half of the newly developed root system. After painting the exposed areas of wood, 2 lb. of hydrated lime were scattered around the base and the sand was again banked around it. The tree soon regained its healthy appearance and has continued to remain healthy. When the bank of sand was washed away in June, 1941, to permit examination, a new growth of healthy roots, developed from the callus formed above where the bark was cut away on the trunk, was found to have filled in the gap left by removing the diseased roots approximately 3 years previously. Thus, by the simple expedient of banking, following surgical treatment, it has been possible to restore this apparently doomed tree to its former health and to continue to maintain it for a period of more than 9 years, despite reinfection by Clitocybe root rot. By so doing, it has been possible to preserve the ornamental value of a row of formally pruned trees in which the loss of a central one would have proved highly objectionable.

It is not known how this tree became infected again, as it was thoroughly treated at the end of 1932, when a small piece of oak root infected by the Clitocybe root-rot fungus was found under it and removed. One other formally pruned Australian pine similarly treated subsequently for Clitocybe root rot also became reinfected after the development of an excellent new root system was induced by banking following surgical treatment, but had declined to such an extent when the disease was observed that re-treatment and banking failed to save it. However, drought was an important factor in its death. Other trees of *Casuarina lepidophloia* attacked by Clitocybe root rot, including both formally pruned and unpruned ones, have been saved, without evidence of reinfection, for periods of several years by surgical treatment followed by banking. Cutting back the tops of treated trees was not necessary, even on unpruned ones.

In resorting to the banking method of treatment to save trees attacked by root diseases, it is advisable to treat the trees before they become girdled. On January 29, 1936, a far-fetched attempt was made to save another formally pruned tree of *Casuarina lepidophloia*, observed to be completely girdled by Clitocybe root rot 2½ months previously. In treating this tree it was necessary to excise the bark entirely around the base of the trunk up to a height of 18 inches on one side and 27 inches on the other. The entire root system was found to be dead from invasion by the fungus, but some roots had to be left to support the tree. A well of bricks about a yard square was laid around the base of the tree and filled with sand to a point well above the highest excised bark. This tree began to shrivel and die by March 15, and

on March 30 the bricks were removed and the bank torn down to examine the extent of new root development. Several masses of tender roots, some as much as 12 inches long, were found to have developed from the lowest margin of callus formation and a few straggling ones higher up on the trunk in the period of two months following surgical treatment and banking. This, of course, was an extreme case and is cited to show the extent of root development that may be expected in a short time, even with the tree nearly dead.

The rejuvenation by banking of attacked trees following surgical treatment is by no means limited to citrus trees and Australian pines. It has been tried with considerable success by the writer and growers whom he has had occasion to advise, on a variety of trees, both large and small, and also on shrubs attacked by Clitocybe root rot. The results here reported have been secured under extremely droughty soil conditions and it is believed that even more favorable results would be secured in soils where favorable moisture conditions prevail. The method appears to be applicable to any woody plant that develops adventitious roots readily.

DISCUSSION

Surgical treatment or aeration, or the use of these in conjunction, is effective in arresting the progress of the disease. However, while such procedure may eradicate the infecting fungus, it does not assist trees handicapped by the loss of a large portion of their root systems to recover. The value of the soil-banking method of treatment is, therefore, apparent, as shown by the examples cited. In addition, it soon reduces or eliminates the possibility of the trees blowing over. It is essentially a natural method of inarching which has proved far superior to the usual method. It is possible that the use of some hormone-like substance to stimulate root development might be desirable, though excellent results have been secured on a variety of trees and shrubs thus far treated without recourse to such agents.

Soil-banking is diametrically opposed to the aeration method for control of root diseases. Since banking tends to favor the continued development of such diseases, it is a wise precaution ordinarily to first eradicate the disease by surgical treatment and disinfection and follow this by brief aeration. This is particularly advisable in the case of the mushroom or toadstool root rots, which are more difficult to control than other root diseases. In the control of foot rot of sweet seedling orange trees, aeration alone has proved very effective, since it appears to attack merely the bark. In many cases the disease appears to run its course after a time but the trees become weakened as a result of invasion by various, purely secondary, wood-rotting fungi. In long-standing cases of this disease on old trees, there are so many dead and rotted roots that no longer harbor the pathogenic organism, and so much decay in the bases of the trees, that painstaking surgical treatment is impracticable. With such trees, which would soon succumb or blow over ordinarily,

the simple expedient of banking has been found to greatly prolong their life and productivity.

SUMMARY

The present well-known methods for the treatment of trees attacked by root diseases are discussed and evaluated.

Attention is called to the usefulness and practical value of a little-known natural method of inarching whereby trees and shrubs extensively girdled by root diseases may be rejuvenated by soil banking, either alone or in conjunction with previous surgical treatment, disinfection, and aeration, to stimulate the development of a new root system above the partially girdled bases.

The results secured by the use of this method on citrus trees and Australian pines are described and illustrated and its applicability indicated for use on woody plants that develop adventitious roots readily.

This soil-banking method offers the only known practical means of saving old sweet seedling orange trees in which the disease appears to have run its course and which have deteriorated to such an extent that painstaking surgical work is not justifiable.

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MOSAIC OF CELERY CAUSED BY THE VIRUS OF ALFALFA MOSAIC

WILLIAM C. SNYDER AND SAUL RICH

(Accepted for publication September 24, 1941)

In 1937 the writers observed that healthy plants of celery (*Apium graveolens* L.), artificially inoculated in the greenhouse with the virus of alfalfa (*Medicago sativa* L.) mosaic, developed a bright yellow mottle of a calico-like mosaic. Since that time, repeated inoculations have been made using alfalfa-mosaic virus of several sources collected from different areas in California, with the same kind of results. The writers make no attempt here to identify this mosaic with celery calico (1, 2), pseudocalico (3), or any other mosaic of celery previously reported, but describe it in the belief that it may be one of those involved in the complex of naturally occurring celery viroses. It is recalled that Black and Price (8) recently demonstrated that calico mosaic of potato may be caused by the virus of alfalfa mosaic.

Zaumeyer (4) apparently did not include celery in his host-range studies of the alfalfa-mosaic virus, nor did Price (5) report any test of this virus on celery in his comprehensive investigation of host ranges.

Four California sources of the alfalfa-mosaic virus were used in producing mosaic of celery in greenhouse studies on the host range of the virus. These inocula were obtained from field specimens of alfalfa growing in the Sacramento Valley, the Salinas Valley, and near Santa Maria and Riverside, respectively. Each of the original infected plants showed typical symptoms of alfalfa mosaic of the type illustrated in figure 1, A. The properties of the virus, determined for one of the collections, was found to be as follows: temperature inactivation point, 60–65° C.; dilution end point, 1/2000–1/3000; longevity *in vitro*, 3–5 days. The aphis transmissibility of the virus was demonstrated by means of the pea aphid, *Illinoia pisi* Kalt.

Infection was obtained by juice inoculations with the aid of carborundum as an abrasive, after the method of Rawlins and Tompkins (9). The amount of transmission obtained was usually low, but in a few trials it ranged as high as 50 per cent or more. Celery of the Golden Self Blanching type was used throughout. The inoculum for these trials was prepared by extracting the juice from *Vicia faba* L., *Petunia hybrida* Vilm., *Trifolium repens* L., and *Melilotus indica* All., respectively, artificially infected in each case by the alfalfa-mosaic virus. Successful transmission of the virus from celery to celery also was accomplished. The recovery of alfalfa-mosaic virus from celery was generally easier than its establishment in celery, and was obtained either systemically or in local lesions by mechanical inoculation of such host plants of the virus as *Medicago sativa* L., *Vicia faba*, *Vigna sinensis* Endl., *V. sesquipedalis* Wight, *Glycine max* Merr., *Petunia hybrida*, *Lathyrus odoratus* L., and *Trifolium repens*. The symptoms of the recovered virus

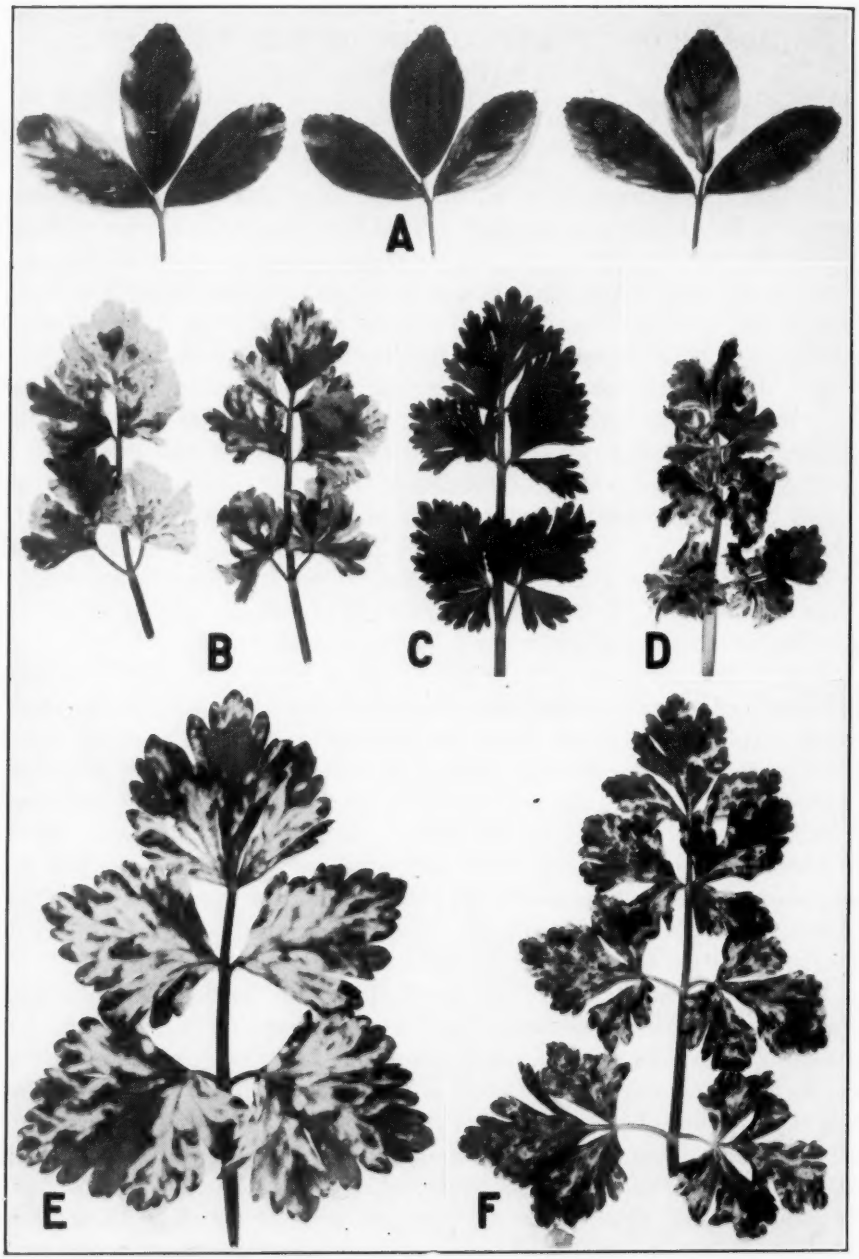

FIG. 1. Symptoms of alfalfa mosaic virus produced by artificial inoculation on common alfalfa, A, and on celery, showing mosaic on outer leaves, B, E, and F, and on an inner leaf, D. The leaf shown at C is from a healthy plant.

on these plants were the same as those obtained before its passage through celery, and, on alfalfa, were typical of alfalfa mosaic (Fig. 1, A).

The symptoms induced by alfalfa-mosaic virus on celery (Fig. 1, B–F) consist of a mild to conspicuous yellow-green mosaic, principally of the outer leaves. In some cases, however, the inner leaves also show symptoms. When the symptoms are most marked, the affected leaf presents a striking calico-like pattern of lemon yellow patches on a normal green background. A mild blister effect often is associated with the occurrence of green islands of tissue in the yellow areas; and, in severe cases, a tendency to leaf distortion or a backward roll of the leaf may be observed. In early stages of the disease, vein clearing or a yellowing of the veins is often apparent. In later stages, yellow or cleared rings and halos may occur, surrounding areas of green tissue.

The appearance of symptoms on inoculated celery was sometimes slow, some inoculations requiring a month before the symptoms were well defined under the conditions obtained in the greenhouse. Sometimes, after 3 or 4 months from the time of inoculation, the symptoms faded out or changed to a general chlorosis, but the virus was still recoverable from such plants. A similar phenomenon was reported by Porter (6, 7) for potato calico and is well known for alfalfa mosaic.

It is suggested that there may be a direct relationship in the field between alfalfa mosaic and the calico-like mosaic of celery described here, especially when celery is grown in the vicinity of diseased alfalfa. Circumstantial evidence in support of this view is found in the observation that a calico-like mosaic frequently is found on celery in areas in which alfalfa is widely grown.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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PHYTOPATHOLOGICAL NOTES

The Perfect Stage of Phomopsis vexans.—The fungus, *Phomopsis vexans* (Sacc. and Syd.) Harter, has been known since 1912[1] to cause one of the most important diseases of eggplant (*Solanum melongena* L. var. *esculentum*). This pathogen and the disease, variously known as tip over, leaf blight, fruit rot, leaf spot, stem blight, and eggplant blight, that it causes have been studied by numerous investigators, among whom were Sherbakoff,[2] Edgerton and Moreland,[3] Nolla,[4] Palo,[5] Toole, E. H. *et al.*,[6] and Howard and Desrosiers.[7] It has never been reported present on any other host plant.

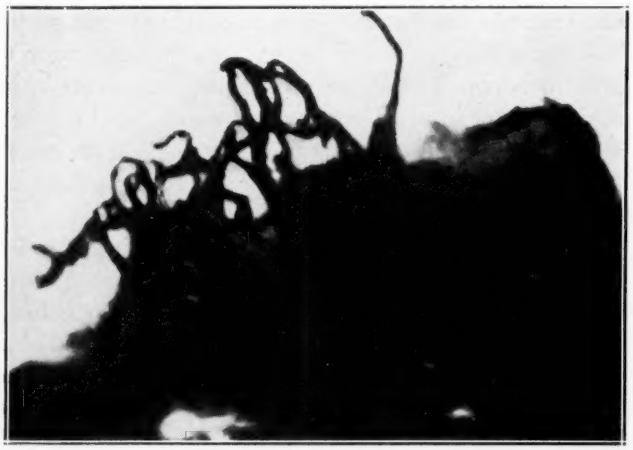

FIG. 1. A stroma bearing clustered, beaked perithecia of *Diaporthe vexans* growing on 2 per cent potato-dextrose agar. ×17.5.

To date the perfect stage of this fungus has not been described, but mycologists have long suspected that eventually it would be included in the genus *Diaporthe*. This is indicated by Wehmeyer as follows: "The imperfect or *Phomopsis* stages of several species of *Diaporthe* are known . . . to be the cause of serious diseases of several plants: for instance, *D. Citri* (*Phomopsis citri*) . . . ; *D. phaseolorum* . . . ; *D. sojae* . . . ; and *D. batatatis*. . . .

[1] Harter, L. L. Fruit-rot, leaf-spot, and stem-blight of the eggplant caused by *Phomopsis vexans*. Jour. Agr. Res. [U.S.] 2: 331–338. 1914.

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[7] Howard, F. L. and Russel Desrosiers. Studies on the resistance of eggplant varieties to Phomopsis blight. Proc. Amer. Soc. Hort. Sci. 39: 337–340. 1941.

In these species the perithecial stage is rarely found or is known only from culture" (p. 3).[8]

In November, 1939, while making comparative studies of several isolates of *Phomopsis vexans* growing on 2 per cent potato-dextrose agar, it was

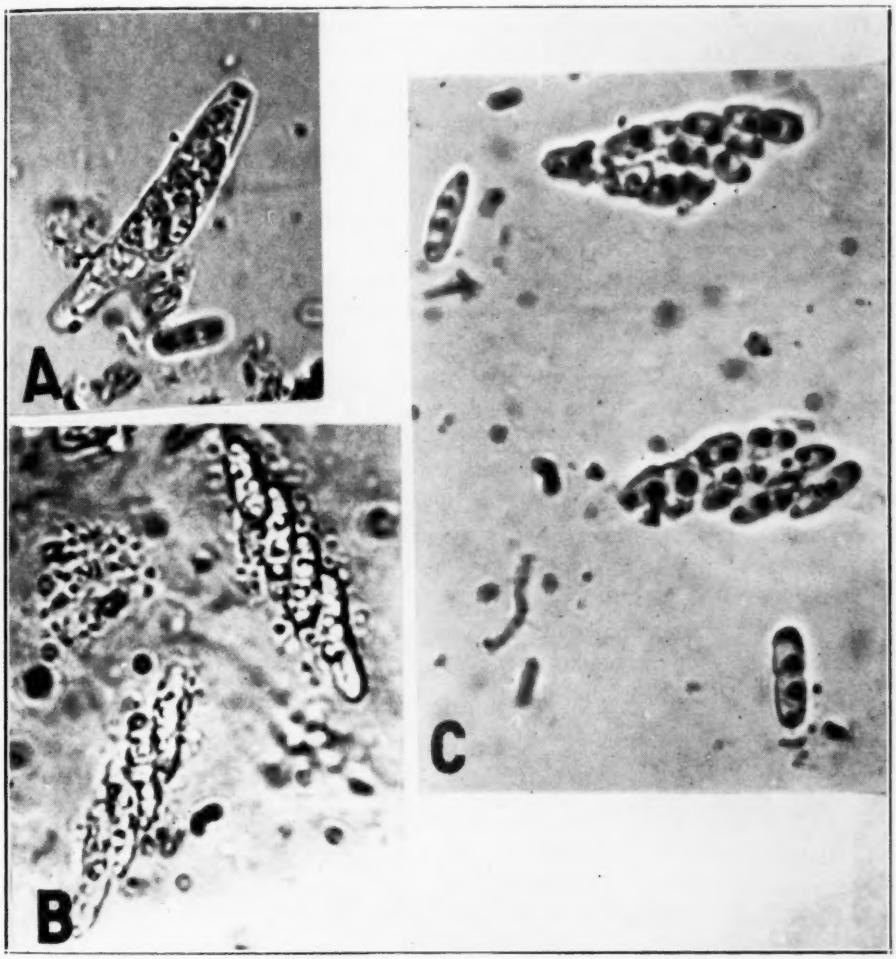

FIG. 2. Asci and ascospores of *Diaporthe vexans* showing: *A*. Apical pore. ×880. *B* and *C*. Guttulae, septation, constriction, bluntness, and arrangement of spores. ×920.

observed that one of these was producing characteristically beaked structures not so superficial as the usual loosely gregarious pycnidia characteristic of other isolates on the same media. About 2 weeks later, and when the cultures were 6 weeks old, perithecia were observed embedded in the carbonaceous stromatous tissue, which was likewise partially embedded in the agar. This was again observed on another isolate in November, 1941.

[8] Wehmeyer, L. E. The genus *Diaporthe Nitschke* and its segregates. Univ. of Michigan Press, Ann Arbor. 349 pp. 1933.

These perithecia, occurring usually in clusters, were from 130 to 350 μ in diameter. The beak-like structures, ostioles, were carbonaceous, sinuate, irregular and from 80 to 500 μ long (Fig. 1). The asci produced in these perithecia were 8-spore, clavate, sessile, 28–44 × 5–12 (av. 36 × 8.9) μ, hyaline, with thin walls, and apex slightly thickened and pierced by a narrow pore. The spores were biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, quite uniform in size, 9–12 × 3.0–4.4 (av. 10.8 × 3.7) μ, bicellular, constricted at the septum with each cell usually containing two guttulae (Fig. 2). In all respects, this fungus, isolated on two occasions from eggplant stems exhibiting characteristic symptoms of "tip over," from Marion County, Florida, is, in appearance, a typical *Diaporthe*. Fifty subsequent single-spore cultures from the same strain produced similar perithecia bearing asci and ascospores identical with those observed earlier.

Numerous lots of eggplant seedlings were inoculated with suspensions of crushed perithecia, or with ascospore suspensions made from the exudate taken from the tip of the elongated ostioles of perithecia, produced in the single-spore cultures just mentioned. Characteristic lesions with typical pycnidia on leaves and stems resulted from these inoculations; these were similar to those produced by parallel inoculations with pycnospore suspensions of *Phomopsis vexans*. Apparently, however, even though numerous, the spots and lesions resulting from inoculations with the perfect stage were not so abundant as those resulting from inoculations with the imperfect stage of the fungus. To date, the perithecial stage of this fungus has not been observed on the host plant.

In view of the evidence herein presented the binomial **Diaporthe vexans** (Sacc. and Syd.) n. comb. is proposed for the perfect stage of the fungus causing the "tip over" disease of eggplant.—L. O. GRATZ,[9] Department of Plant Pathology, Agricultural Experiment Station, University of Florida, Gainesville, Florida.

Influence of Temperature on the Expression of Big-vein Symptoms in Lettuce.—Big Vein, a disease of lettuce, was first described and named by Jagger and Chandler[1] who found it causing some loss in the Imperial Valley of California. While they stated that the cause of the disease was unknown, their observations indicated that the causal factor was soil-borne and persisted for extended periods in field and greenhouse soils. Since the appearance of their paper, no further investigations of the disease have been published.

In visiting lettuce fields in states along the Atlantic Seaboard during the last five years, the senior writer has observed in New Jersey, Maryland, and North and South Carolina what appeared to be big vein, and it seems probable that it may occur in other lettuce-growing sections in the East. At

[9] The writer is indebted to Erdman West, Mycologist, Florida Agricultural Experiment Station, for helpful suggestions relative to this work.

[1] Jagger, I. C., and Norman Chandler. Big vein of lettuce. Phytopath. 24: 1253–1256. 1934.

Beltsville, Maryland, the disease has frequently been noted in experimental lettuce plantings in both field and greenhouse.

During the fall and winter of 1939–40, big vein appeared on a number of lettuce plants in the greenhouse at Beltsville, where the development of the symptoms was such as to indicate that temperature definitely affects manifestation of the disease. The group of lettuce plants in which the disease

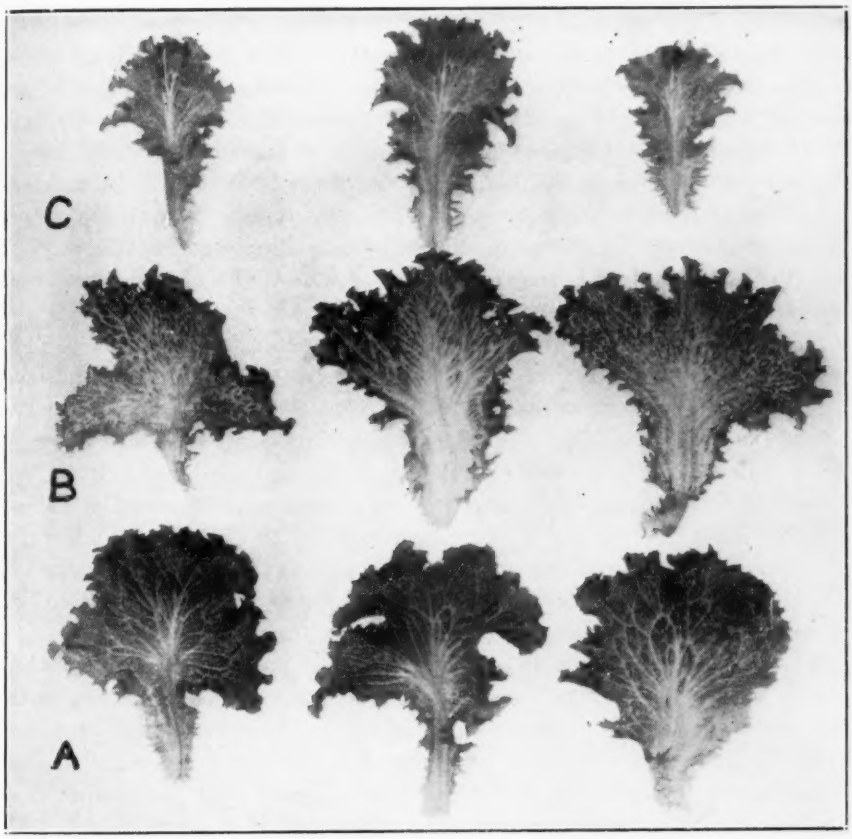

FIG. 1. Effect of temperature on expression of big-vein symptoms in lettuce. A. Leaves from base of seed-stem produced at fairly high temperatures (day, 65° to 75° F.; night, 50° to 60° F.) and showing no evidence of disease. B. Leaves from segment of stem directly above *A* produced at lower temperatures (day, 50° to 60° F.; night, 45° to 50° F.). These show pronounced big-vein symptoms. C. Leaves grown on segment of stem above B at same temperatures as A. Note that symptoms are again suppressed. (Photographed by transmitted light.)

appeared was grown in 10-inch clay pots and held in a section of the greenhouse where, during the day, the temperature was originally maintained between 65° and 75° F., while at night it ranged from 50° to 60°. Occasionally the day temperature exceeded 75°, but only for short periods. When the lettuce plants had reached the stage of seed-stem elongation, it was necessary to reduce the day temperature to 50° to 60° and the night temperatures to 45° to 50°, because of the requirements of other experimental material

grown in the same greenhouse section during this period. The temperatures thus maintained for 3 weeks were then raised to the original day and night ranges, and there held as nearly as possible until the plants reached maturity.

At the time the temperature was lowered all the plants were large and vigorous, of good color, and the leaves showed no evidence of disease (Fig. 1, A). At this time the seed stems of most of the plants had reached a height of 12 to 15 inches. During the succeeding 3 weeks at the lower temperatures, these stems made an additional growth of 8 to 12 inches. In certain of the plants all of the new leaves produced on that portion of the stems developed during the low-temperature period showed an enlargement of the vascular regions of the petiole and leaf blades. These areas were almost devoid of chlorophyll, and, when examined by transmitted light, a pronounced vein-banding was evident throughout the leaf. All of the affected leaves also were abnormally savoyed. These symptoms (Fig. 1, B) are all typical of the big-vein disease as described and illustrated by Jagger and Chandler[1] and it would appear that the plants showing such symptoms were infected before the temperatures were lowered.

When the greenhouse temperatures were again raised to 65° to 75° F. during the day and to 50° to 60° F. at night, the leaves first produced on those portions of the stem that developed after the temperature was raised showed some faint evidence of the vein-banding and savoying described above. As more leaves were produced, however, the later growth was normal in appearance and could not be distinguished from the foliage formed during the previous high-temperature period (Fig. 1, C). None of these later leaves showed any evidence of big-vein symptoms during the life of the plant, although the symptoms remained unchanged in the leaves of the low-temperature period.

These observations seem to indicate that the expression of big-vein symptoms in lettuce is greatly influenced by the air temperature prevailing at the time the leaves are in process of development. This belief has been confirmed by other observations on plants grown in the greenhouse and field at Beltsville, and is also supported by Jagger's report[1] that the symptoms of the disease were more pronounced and the percentage of diseased plants was possibly higher in crops grown in the Imperial Valley during the fall and winter months.—ROSS C. THOMPSON and S. P. DOOLITTLE, U. S. Horticultural Station, Beltsville, Maryland.

Sorghastrum, Host of an Undescribed Smut.[1]—The genus *Sorghastrum,* Indian grass, is abundantly represented throughout Virginia by *Sorghastrum nutans,* (L.) Nash. The long-awn Indian grass, *S. elliottii,* (Mohr) Nash, however, has been reported only in the Coastal Plain.

While on a fall collecting trip for vascular plants the senior writer found a small colony of *Sorghastrum* that showed a high incidence of head smut.

[1] Cooperative Wildlife Research Unit, Dept. Biology, Virginia Poly. Inst. and Department of Plant Pathology, Agri. Extension Service, Penn. State College.

The location of the colony being in the Piedmont province, 125 miles west of the Coastal Plain, little attention was given to the species of the host. With attention focused on the infection, the host was arbitrarily accepted as *S. nutans*. Later, on examining the host, it was definitely identified as long-awn Indian grass, *Sorghastrum elliottii*, and thus the known range of the host species in Virginia was extended. The frequency of the occurrence of *S. nutans* over the State in contrast to that of *S. elliottii* probably explains why the smut had not been found previously, and especially, since host specificity is indicated. Host specificity is further indicated by the subsequent discovery of the disease on the same host in Georgia.

From the general appearance of the infection and the close relation of the host to *Sorghum*, the field impression was that of sorghum smut on a new host. When visiting Washington a few days later the material was shown to C. L. Shear and J. A. Stevenson of the Division of Mycology and Disease Survey. They expressed the opinion that *Sorghastrum* was a rare host for smut and that the smut probably was an undescribed species. Stevenson sent some of the material to the junior writer who reported that he not only considered *Sorghastrum* a rare host for smut but that the fungus was an undescribed species of *Sphacelotheca*. He was then asked to prepare a description of the new species for inclusion in this report. The name *Sphacelotheca sorghastri* is here proposed and described as follows:

Sphacelotheca sorghastri Zundel nov. sp.

Sori destroying the inflorescence, long linear, 7–10 cm. long, 3–4 mm. wide, at first protected by the leaf sheath, covered by a thick, yellowish membrane that flakes away, exposing a dark-brown, semi-agglutinated spore mass surrounding a compound columella of 7 or more branches extending the length of the sorus; membrane disintegrating into sterile cells, singly, in chains or in groups, globose to subglobose or ellipsoidal, flattened by mutual contact, hyaline, chiefly 7.5 to 14 μ diameter, vacuolated, thin epispore; spores globose to subglobose, regular, light olivaceous-brown, chiefly 7.5 to 10.5 μ diameter, abundantly and minutely echinulate, thin epispore.

On *Sorghastrum elliottii* (L.) Nash, Moses Mill Pond, 2 miles west of Chatham, Pittsylvania Co., Virginia. Coll. A. B. Massey. 5059 Sept. 5, 1941.

Latin diagnosis by Edith K. Cash of the Bureau of Plant Industry, U.S.D.A.

Sphacelotheca sorghastri Zundel nov. sp.

Sori inflorescentias destruens, longe lineares, 7–10 cm. longi, 3–4 mm. lati, primum vagina folii inclusi, membrana crassa flavida tecti; membrana frustulatim defrigens et massam sporarum nigrobrunneam, subagglutinatam denudans; columella e ramis 7 vel pluribus per longitudinem totam sori extensis compositis; membrana in cellulis sterilibus singularibus, catenulatis, vel aggregatis, globosis, subglobosis vel ellipsoideis, pressione inter se applanatis, vacuolatis, episporio tenui disrumpens; sporae globosae vel subglobosae, regulares, pallide olivaceo-brunneae, plerumque 7.5–10.5 μ diam., dense et minute echinulatae, episporio tenui.

In florescentiis *Sorghastri elliottii* (Mohr) Nash, prope Chatham, Virginia.

Since studying the material and describing the fungus as a new species, Zundel has received a specimen of the same smut on *S. elliottii* from Georgia. This was collected by Messrs. J. H. Miller and G. E. Thompson at Camp Willkins, Athens, Georgia, on October 14, 1941, thus indicating that the new species of *Sphacelotheca* is well distributed with its specific host.

The two species of *Sorghastrum* reported in Virginia are of little or no

economic importance. In the Great Plains *Sorghastrum nutans* is an important constituent of tall-grass hay and is considered rather nutritious. In Virginia, however, it is not recognized as a hay grass. It occurs throughout the State, but usually only in small clumps or patches. The most extensive natural field development of this species has been observed in the Triassic area of northern Virginia, especially in Prince William and Fauquier Counties, where it occurs in fields of considerable acreage. It also has been observed on banks and slopes in several places in the State, effectively checking erosion. *S. elliottii*, on the other hand, has been reported previously in only a few Coastal Plain counties in Virginia; hence, it is of even less importance. The Sorghastrum smut, therefore, would be classed as of negligible economic importance. It does, however, present an attractive problem in the biology of a smut of a perennial grass.—A. B. MASSEY AND GEORGE L. ZUNDEL, Virginia Polytechnic Institute, Blacksburg, Va., and Pennsylvania State College, State College, Pa.

A Method of Mounting Cultures of Fungi for Preservation in the Herbarium.—It has been the custom in various institutions to file as herbarium specimens Petri-dish agar cultures of fungi. The agar removed from the dish is dried down on a sheet of paper or cardboard and then placed in a herbarium packet. This method has, at least, two disadvantages: (1) the agar sheet curls and shrivels, on drying, often becoming cracked or otherwise damaged; (2) the cultures thus mounted cannot be observed advantageously under the microscope by transmitted light.

Recently the writer has found that celluloid makes a more satisfactory mounting base than does paper or any of the other materials known to have been utilized previously for this purpose. Though the procedure followed in making such mounts is simple, it is outlined in detail below.

The celluloid[1] after being washed in soap and water, is rinsed, dried, and cut into squares of a diameter exceeding slightly that of the Petri dish. The agar medium with its mycelial mat[2] is lifted from the Petri dish with a spatula, laid in the center of the celluloid square, and allowed to dry. Within 3 days at room temperature ranging from 20° to 25° C., the agar is sufficiently dry to permit filing the mount in a herbarium packet.

The agar becomes firmly attached to the celluloid and, thereafter, will withstand a remarkable amount of twisting and bending. Mounts prepared in this manner were stored at relatively high temperatures (30–40° C.) for 10 days without the cultures cracking or peeling away from the base. However, the whole culture may peel off if the fungus is allowed to grow to the edge of the dish before being mounted. It is best to make the mount while there is still a peripheral strip of uncontaminated agar, at least $\frac{1}{2}$ in. wide, around the thallus. If it is desirable to mount older or larger cul-

[1] Obtained from the DuPont Viscoloid Co., Arlington, New Jersey, with the following specifications: 0.01 in. thick, transparent, color 7511, and HH finish.

[2] The fungi (*Stemphylium* sp., *Cephalosporium* sp., and *Colletotrichum* sp.) were growing on 2 per cent potato-dextrose agar, poured to a depth of approximately $\frac{1}{4}$ in. in the Petri dishes.

tures, it may be necessary to apply first an adhesive or a fixative to the celluloid.

The cost of such celluloid bases is estimated at slightly less than one cent each (50 cents per sheet measuring 20″ × 50″).

Cultures preserved on celluloid can be examined by transmitted light under a cover slip if a drop of water, lactic acid, or other mounting fluid first be placed on the specimen. The gross characteristics, *e.g.*, zonation, chromogenesis, etc., can be studied almost as well as in the fresh condition.—M. B. LINN, Department of Plant Pathology, Cornell University, Ithaca, New York.

AMERICAN PHYTOPATHOLOGICAL SOCIETY
SUMMER MEETING

SECOR HOTEL, TOLEDO, OHIO

JUNE 25–26, 1942

In the rapidly changing times participation in war emergency meetings should be encouraged. The summer meeting of The American Phytopathological Society is definitely a war emergency meeting. It will deal with "The role of plant pathologists in a war emergency program." Emphasis will be placed on activities of the Society's War Emergency Committee. Everyone will be intensely interested in the progress made by this committee, and in assisting in formulating future activities.

Attend the summer meeting and be informed.

TENTATIVE PROGRAM

Secor Hotel
Toledo, Ohio

June 25

10:00 a. m. The role of plant pathologists in the war program. Summarized by the War Emergency Committee, E. C. Stakman, Chairman.

1:30 p. m. Round-table discussion on spray-material and spray-equipment priorities and substitute materials. This will include reports by members of the Society and representatives of industry. J. S. Horsfall, Chairman.

3:30 p. m. Demonstration of techniques used in determining physical properties of dust mixtures and performance of dusting equipment. Co-chairman, J. D. Wilson, Ohio Experiment Station, and Frank Irons, U. S. D. A., Engineering Laboratory.

7:30 p. m. Opportunity for regional or special committee meetings.

June 26

10:00 a. m. Discussion on extension, research and teaching policies during present emergency.—Leaders: H. B. Barss, O. D. Burke, N. E. Stevens.

1:30 p. m. Summary of program, policies and future activities of the War Emergency Committee. (Members of the executive committee in charge.)

Opportunity will arise in various sessions for a discussion of quarantines, the draft, disease surveys, and other subjects that the War Emergency Committee, or members desire.

An extensive list of Institutions, Societies, and Research Workers in the pure and applied plant sciences in C. and S. America has been prepared by the Editors of Chronica Botanica, in cooperation with the Div. of Agriculture of the Office of the Coordinator of Inter-American Affairs, Washington, D. C. It has been published in Chronica Botanica, Vol. 7, no. 2 and 3 (March and May, 1942).